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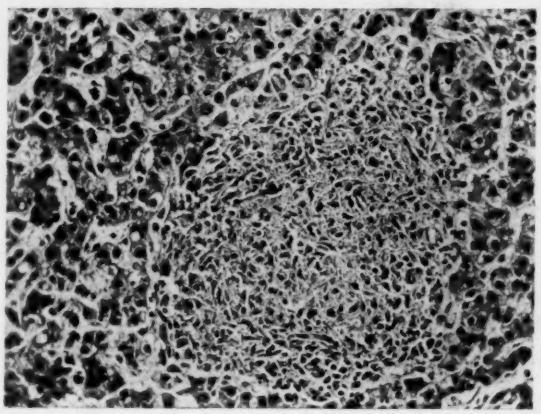

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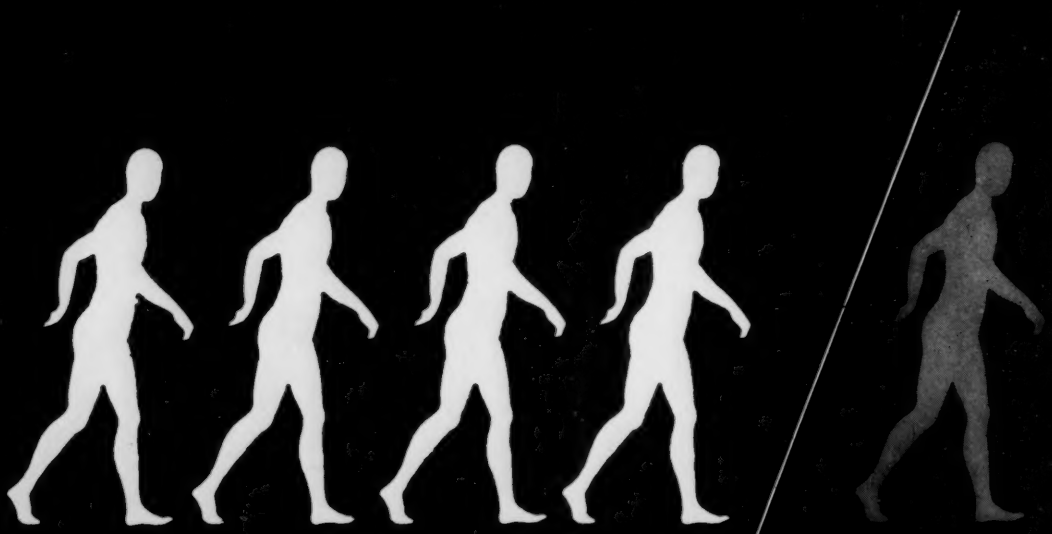

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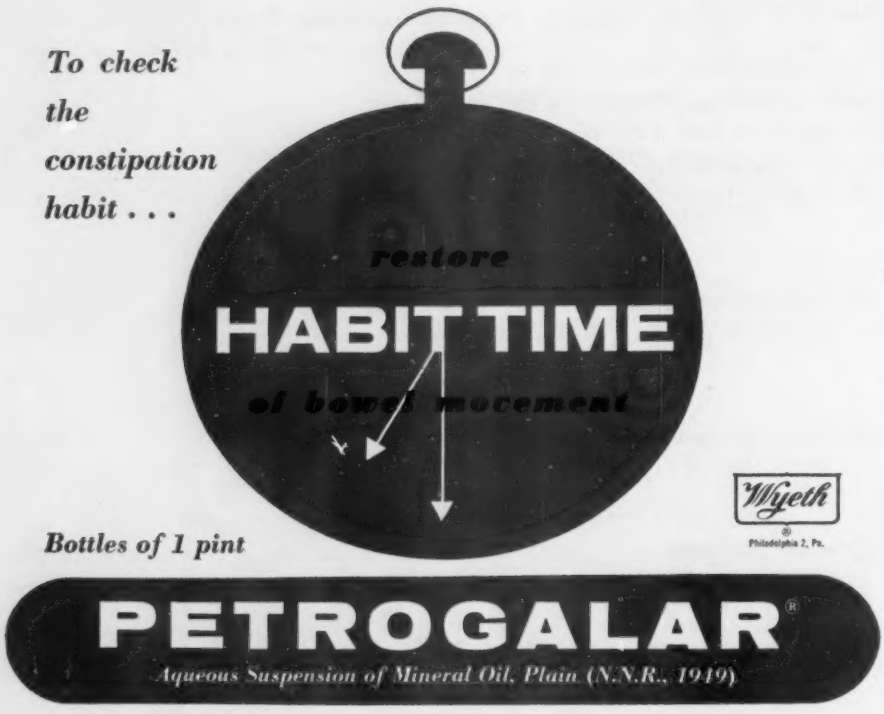

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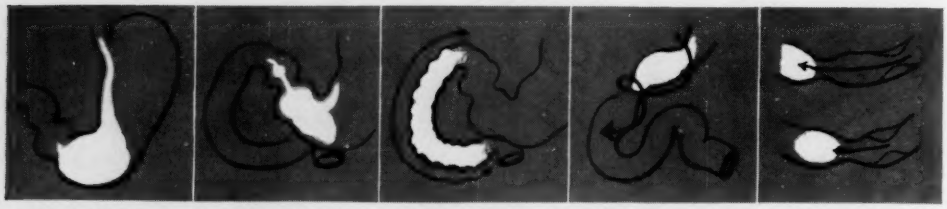

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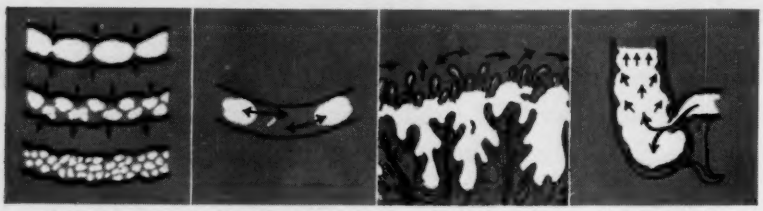

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SEARLE

Epidemic of Infectious Hepatitis

Some Notes on Delineation of High-Risk Groups and Protection of Exposed Susceptibles by Gamma Globulin

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Studies on the viral hepatitides of man have aroused increasing interest in the past few years, and several studies of epidemics of infectious hepatitis have been recorded.* The present report is concerned with a brief review of the literature pertinent to this problem and with recording an epidemiological study, with particular reference to delineation of high-risk groups in an open community outbreak. In addition, some observations on the effect of normal human serum gamma globulin in protecting exposed susceptibles under field conditions will be presented, and the proper application of this material in open community outbreaks will be considered.

Present address: Department of Medicine, The New York Hospital-Cornell Medical Center (Dr. Barondess); Research Fellow, National Institutes of Health, U. S. Public Health Service (Dr. Bashe).

This investigation was conducted in part under the sponsorship of the Commission on Liver Diseases, Armed Forces Epidemiological Board, and was supported in part by the Office of the Surgeon General, Department of the Army.

From The Children's Hospital of Philadelphia and The Department of Pediatrics, School of Medicine, University of Pennsylvania, Philadelphia; The Communicable Disease Center, Public Health Service, Department of Health, Education, and Welfare, Atlanta; The Dalton-Whitfield County Health Department, Dalton, Ga., and Division of Epidemiology, Georgia State Department of Public Health, Atlanta.

* References 1-14, 17, 21, and 43.

REVIEW OF THE LITERATURE

Infectious hepatitis is a very old and common disease and has no doubt been responsible for many of the cases which physicians have been identifying as "catarrhal jaundice" for many years. Cockayne [42] cites Cleghorn's report of what was probably an outbreak of infectious hepatitis on the Island of Minorca in 1745. Lucke [15] has reviewed the frequent occurrence of the disease in armies in the Franco-Prussian War, the American Civil War, World Wars I and II, and others. Blumer [16] recorded 200 separate outbreaks during a one-year period (1921-1922) in New York State alone.

Additional evidence of the frequency of infection with the virus of this disease may be found in three facts: 1. Pools of plasma from normal adults in this country, when fractionated by the techniques of Cohn and his co-workers,[31] provide gamma globulin which apparently contains high and relatively constant titers of protective antibodies against infectious hepatitis, when large pools prepared on a national basis are used. Thus, one may infer that a relatively large proportion of American adults have had antigenic experience with the virus; further, the fact that only a few adults give a recognizable history of jaundice indicates that many of these infections have been anicteric, and many of these were no doubt subclinical. 2. A skin test for infectious hepatitis, developed by the Henles and their co-workers,† when applied to a group of normal persons with no known history of infectious hepatitis, was read as positive, indicating past infection with the virus, in 30 to 40% of adults and 10% of children tested. 3. Studies of naturally occurring epidemics of the disease have shown that

† References 32-34.

the age-specific incidence of infectious hepatitis falls off rapidly after the age of 20 years ‡; it appears most likely that this primarily reflects immunity due to prior infection with the virus, whether or not such infection was accompanied by a clinically apparent illness.

The latter two points suggest that, at least insofar as endemic infectious hepatitis is concerned, the anicteric form may be considerably commoner than the icteric disease.

Prior studies on the epidemiology of infectious hepatitis have recorded instances in which outbreaks seemed to have common sources in milk,[2] water,§ food,|| or syringes.[9] The disease appears to spread more commonly, however, by person-to-person contact, and epidemics in which this was apparently the case have been reported from both military ¶ and civilian # populations. It has been felt by some * that respiratory spread might be the mechanism involved in the transmission of the disease from person to person, while others † have believed that the intestinal-oral route was more likely the mode of spread. The weight of evidence appears to favor the latter view. Thus, except for two studies,‡ the results of which have not been substantiated by others,§ attempts to transmit the disease to human volunteers by spraying of acute-stage nasopharyngeal washings have failed. On the other hand, virus has been demonstrated in the stools of acute cases || and convalescents,[51] and the disease has been transmitted to humans by feeding fecal suspensions from such persons. Epidemiological studies in institutions # have produced strong evidence against respiratory spread, and, as mentioned above, common-source outbreaks with obvious oral routes of infection have been reported.

Warren,[46] after an extensive review of the epidemiologic literature, has concluded that there is no striking evidence for respiratory transmission and that infectious hepatitis is, for epidemiologic purposes, an enteric disease. Prior studies * have established the fact that the younger age groups are primarily involved in epidemic hepatitis and that the disease is usually relatively mild in young children †; secondary attack rates among family contacts of cases have been noted to be high.‡ The proportion of anicteric cases has varied widely in various epidemics, and it was 90% in the syringe-borne outbreak reported by Capps and his co-workers.[9] Epidemics in open communities have run their courses in periods ranging from a few months to more than a year. Over-crowding and poor sanitation have apparently increased the ease of transmission of the disease.§ In general, in the United States as well as in other parts of the world, the disease has shown a distinct seasonal prevalence, acquiring epidemic proportions in the late fall and winter and declining in the spring.|| The possibility of transmission by an insect vector has received consideration,¶ but experimental evidence on this point is restricted to one negative study in human volunteers, using flies trapped at the site of a camp epidemic.[3]

It has been shown in institutional epidemics # and in army [17] and in camp [28] out-

‡ References 21 and 43; Clark, W.: Personal communication to the authors; Williams, H.; Drake, M. E.; Barondess, J. A.; Maley, V.; McCroan, J., and Murphy, W. J.: Unpublished data; Knight, V., and Drake, M. F.: Unpublished data.

§ References 3-6.

|| References 7 and 8.

¶ References 10 and 17.

References 12-14.

* References 10 and 21.

† References 12-14 and 46.

‡ References 18 and 41.

§ References 3, 19, and 20 and Drake, M. E.; Barondess, J. A.; Bashe, W. J., Jr., and Stokes, J. Jr.: Unpublished data.

||References 3, 18-20, 35, 36, and 38-40.

References 12-14.

* References 16, 21-24, and 43 and Williams, H.; Drake, M. E.; Barondess, J. A.; Maley, V.; McCroan, J., and Murphy, W. J.: Unpublished data.

† References 12-14, 22, and 23.

‡ References 24-26 and 43; Williams, H.; Drake, M. F.; Barondess, J. A.; Maley, V.; McCroan, J., and Murphy, W. J.: Unpublished data; Knight, V., and Drake, M. E.: Unpublished data.

§ Reference 22 and Williams, H.; Drake, M. E.; Barondess, J. A.; Maley, V.; McCroan, J., and Murphy, W. J.: Unpublished data.

|| References 10 and 22.

¶ References 3, 22, 36, and 46-48.

#References 12-14, 27, and 29.

breaks that human gamma globulin from normal adult donors protects against infectious hepatitis in doses as small as 0.01 cc. per pound of body weight, when given more than five to seven days before the appearance of symptoms. In institutional outbreaks this protection has been shown to last for relatively long periods of time, up to nine months.[27] Thus, with the availability of an effective prophylactic, one of the pressing problems in the field becomes the proper application of this material in affected communities. It would seem that such technique should be based upon careful delineation of high-risk groups in epidemic areas, and identification of such groups must proceed from epidemic studies.

THE EPIDEMIC

From June, 1951, to September, 1952, an epidemic of infectious hepatitis occurred in Dalton and Whitfield County, Ga., totalling 335 reported cases. The epidemiologic study which was done is summarized in the following pages.

Methods.—Practicing physicians in the epidemic area were contacted and apprised of the purposes of the study. They were requested to report all cases of infectious hepatitis to the Health Department by card or telephone. All cases were then visited by a physician-epidemiologist or a nurse-epidemiologist, and detailed data concerning the illness were collected on a prepared form. These included onset date; symptoms and signs; history of injections within six months; antecedent, coincident, or subsequent cases in the family or environment; animal and insect contacts; food, water, milk, and ice cream supplies, and school or occupation. Data were analyzed directly from these forms.

Methods used in the human volunteer studies at The Children's Hospital of Philadelphia have been previously reported, as have techniques used in the various liver-function tests.[30]

It is estimated that case-finding and reporting were of a high degree of accuracy, as far as cases of hepatitis with jaundice were concerned. In the absence of a widespread survey of the population in general, and of family contacts of cases in particular, with liver-function tests, cases of anicteric hepatitis were no doubt missed, but gross trends based upon the available cases should hold good in evaluating the chief patterns of spread of the virus through the community. Analysis of the data in this report is based on all known cases except where specified otherwise.

Attack rates are based on U. S. Census figures for 1950.

The Area.—The city of Dalton, Ga., is the county seat of Whitfield County, in Northwest Georgia, some 93 miles from Atlanta. It lies in the Appalachian Valley, in a limestone-substrate area. Whitfield County is primarily a farming region, while the chief industries in Dalton are chenille manufacture and chicken raising. The chicken flocks are generally in good condition, with no serious epizootic problems. Fly, mosquito, rat, and mouse populations are not unduly large; tuberculosis is the chief public health problem. Local medical facilities are excellent. Fairly strict segregation of the Negro population is practiced in this area.

City water supply and sewage disposal facilities are good, but most homes in the areas outside the city have dug wells and outdoor privies or septic tanks.

Endemic viral hepatitis has not been a problem in the area. A few of the practicing physicians recalled having seen an occasional case of infectious hepatitis in the region in the past few years, but only two such apparently endemic cases were found in the year prior to the outbreak. There have been no documented cases of serum hepatitis in the area in the past several years.

Clinical Picture.—The typical case in this outbreak was of moderate severity, beginning with anorexia, nausea, vomiting, general malaise, and headache. After 1 to 10 days, but usually after 2 or 3, there appeared darkening of the urine, lightening in the color of the stools, and gross jaundice. Low-grade fever was common at onset. Most of the illnesses lasted one to three weeks. The incidence of various symptoms and signs in the

TABLE 1.—*Incidence of Various Symptoms and Signs in 297 Cases of Infectious Hepatitis**

Symptom or Sign	Cases	
	No.	%
Jaundice	282	95
Nausea	279	94
Weakness	276	93
Abdominal pain	273	92
Dark urine	263	89
Vomiting	253	85
Anorexia	251	84
Headache	228	77
Light stools	159	54
Diarrhea	106	36

* Fever and hepatomegaly have been omitted in this summary because these data were collected from patients directly, and not from physicians; hence estimates of the incidence of fever and hepatomegaly would be unreliable.

297 cases in which these data were adequate is summarized in Table 1.

The striking difference between the number who noted a darkening of the urine and those who noticed lightening in the color of the stools may be more apparent than real, since many persons did not observe the stool color, whereas color of the urine was usually noticed.

Fifteen patients (4.5% of the total) were classified as cases of anicteric hepatitis, on the basis of anorexia, nausea, vomiting, and dark urine and/or light stools; some had abdominal pain. The proportion of anicteric cases is probably somewhat higher than these figures indicate, as mentioned above.

Laboratory studies revealed hyperbilirubinemia, choluria, and normal leucocyte counts in the patients for whom they were done. Agglutinations against Leptospira icterohaemorrhagiae, L. pomona, and L. canicola were negative in four patients for whom they were done.

There was one death in the series (Case 1), a 34-year-old white woman who died nine weeks after onset, having gone progressively downhill to hepatic failure. The case fatality rate for the epidemic was thus 0.3%.

Epidemic Curve.—The last known endemic case in the area occurred in November, 1950.

TABLE 2.—*Urban and Rural Month-Specific Attack Rates*

Month	Urban Cases, No.	Urban A.R.*	Rural Cases, No.	Rural A.R.*	Total Cases, No.	Total A.R.*
June, 1951	2	0.2	0	0	2	0.1
July	2	0.2	0	0	2	0.1
August	7	0.7	0	0	7	0.3
September	10	0.9	4	0.3	13	0.5
October	20	1.9	22	1.4	43	1.7
November	32	3.0	43	2.7	75	2.9
December	29	2.8	61	3.9	90	3.5
January, 1952	25	2.4	37	2.4	61	2.3
February	6	0.6	7	0.4	14	0.5
March	6	0.6	7	0.4	13	0.5
April	0	0	2	0.1	2	0.1
May	0	0	1	0.1	1	0.04
June	1	0.1	4	0.3	5	0.2
July	2	0.2	1	0.1	3	0.12
August	2	0.2	0	0	2	0.1
September	2	0.2	0	0	2	0.1
Totals	146	14.0	189	12.3	335	12.8

* Attack rate per 1,000.

TABLE 3.—*Age- and Sex-Specific Attack Rates, White Population**

	White Female Cases	White Female Population	Attack Rate†	White Male Cases	White Male Population	Attack Rate†	Total White Attack Rate†
Under 1	0	207	0	0	265	0	0
1-	22	1056	20.8	29	1036	28.0	24.4
5-	89	1418	62.8	65	1348	48.3	55.6
10-	37	2725	13.6	51	2661	19.1	16.4
20-	17	2458	6.9	7	2197	3.2	5.2
30-	3	1813	1.7	5	1667	3.0	2.3
40-	0	1197	0	1	1203	0.8	0.4
50-	1	879	1.1	0	841	0	0.6
60 & over	3	841	3.6	2	830	2.4	2.9
Totals	172	12,594	13.7	160	12,048	13.3	13.5

* The three Negro cases in the epidemic have been omitted, and the calculations are based upon the white population only.

† Attack rate per 1000.

Since that time, case onsets have been as indicated in Table 2. Figure 1 summarizes the monthly changes in the urban and rural attack rates.

The urban origin of this epidemic and its centrifugal spread to the surrounding rural areas of the county are apparent from these data. The relative speed and ease with which this spread was accomplished would seem to reflect the free traffic between the rural areas and the city of Dalton, the relatively high degree of susceptibility of the population, and the relative prevalence of virus in the community.

Age-Sex Incidence.—Selection of younger age groups was observed in this epidemic, as

it has been in others. Table 3 presents these data. Of particular interest is the fact that, although the male attack rates were higher than the female in both the 1-to-5- and 10-to-20-year age groups, the female rate was strikingly higher than the male in the age group 5 to 10. Lilienfeld and his co-workers [24] noted the same phenomenon in an outbreak in Baltimore in 1951. We are unable to explain this difference; it might perhaps be speculated that prepubertal physiological changes, associated with the earlier appearance of puberty in females, increase susceptibility in some way. It is of interest, in connection with this difference in incidence, that sex differences in susceptibility to toxic [44] and dietary [45] liver injury in animals have been reported, and fatality rates in pregnant [49] and postmenopausal [50] women have been found to be high in epidemics of infectious hepatitis.

Another feature of interest is the rise in attack rate in the group of 60 years and over, compared with the rates for those in middle life. This increase might reflect the influence of unrelated chronic or debilitating diseases or perhaps an impairment of anamnestic antibody formation at this age, although the fact that this rise in rate is based on only five cases in the "60 and over" age group makes theorization hazardous.

Racial Incidence.—There were three cases among the Negro population of Whitfield County during the epidemic. These were in a 15-year-old boy, with onset in November, 1951; a 6-year-old girl, with onset in January, 1952, and a 7-year-old girl, with onset in June, 1952. All three children attended the one Negro school in the county. The spacing of onsets indicates that these infections had their source in sporadic contacts with cases in the general population of the community.

Table 4 compares the attack rate among Negroes with the over-all rate for whites.

The marked discrepancy probably reflects primarily the segregation of the Negro population in the area, with corresponding diminution in the degree of contact with infectious persons available to Negroes, as compared with that available to whites. In addition, reporting of Negro cases may have been somewhat less efficient than reporting of white cases, owing to the greater difficulty in recognition of jaundice in Negroes and to a somewhat poorer level of medical care. The possibility that there is a true racial difference in susceptibility to infection with

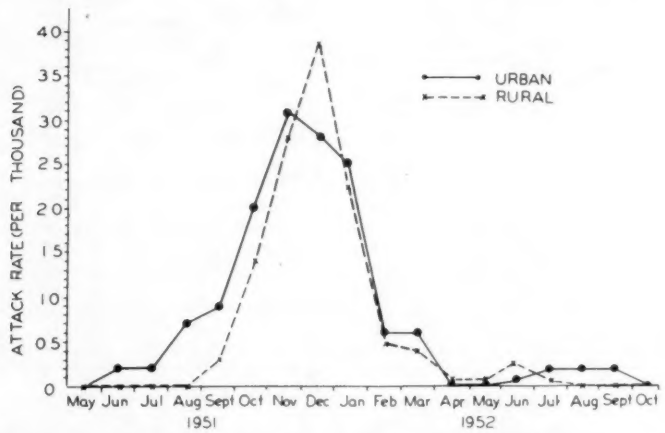

Fig. 1.—Infectious hepatitis in Whitfield County, Ga. Month-specific attack rates by residence.

TABLE 4.—*Race Incidence*

	Population	Cases, No.	Attack Rate*
White	24,642	332	13.5
Negro	1463	3	2.1
Totals	26,105	335	12.8

* Attack rate per 1000.

this virus is not borne out by studies in human volunteers, among whom attack rates are about the same in Negroes and in whites.*

Geographic Incidence.—As noted in Table 2 and Figure 1, for the first three months of the outbreak cases occurred in the urban area only; beginning in September, 1951, rural cases appeared chiefly within small concentrations of population in the County outside Dalton, and in rural schools (*v. i.*). Travel between Dalton and these areas is frequent and fairly heavy, providing ample opportunities for the seeding of these places with hepatitis virus.

Table 2, which summarizes the urban and rural month-specific attack rates, demonstrates that, once the disease became established in the rural sections, attack rates were approximately equal.

TABLE 5.—*Age Incidence by Place of Residence**

	Rural			Urban		
Age, Yr.	Cases, No.	Population†	A. R.‡	Cases, No.	Population†	A. R.‡
Under 1	0	300	....	0	172	....
1-	29	1405	20.6	22	687	32.0
5-	88	1875	46.9	64	891	71.8
10-	56	3513	15.9	34	1873	18.1
20-	8	2634	3.0	16	2021	7.9
30-	6	2047	2.9	2	1433	1.4
40-	0	1414	....	1	986	1.0
50-	1	1073	0.9	0	647	....
60 & over	1	1074	0.9	4	597	6.7
Totals	189	15,335	12.3	143	9307	15.4

* The three cases in Negro patients have been omitted.
† White population.
‡ Attack rate per 1000.

Breakdown of the age data by place of residence reveals, in general, somewhat higher rates among the urban population, especially in the 1-to-5- and 5-to-10-year age groups (Table 5). These data must be interpreted with caution, since there may have been defects in reporting of rural cases; persons living in the more remote sections of the County may not have sought medical advice as readily as those living in more central locations, and some rural dwellers living on the edges of Whitfield County may have gone to physicians in adjacent counties.

School Incidence.—There are 17 schools in Whitfield County, and during the course of the epidemic all became involved. Fifteen of these are primary schools; 1 is a high school, and 1 is both primary and secondary school for Negro children.

TABLE 6.—*School-Specific Attack Rates*

Urban Schools				Rural Schools			
Name	Pupils, No.	Cases, No.	A. R.*	Name	Pupils, No.	Cases, No.	A. R.*
Dalton High	728	5	6.9	East Side	639	27	42.4
North Dalton	361	12	33.2	Pleasant Grove	624	27	43.4
Crown Point	147	9	61.1	West Side	590	10	16.9
Boylston Crown	90	5	55.5	Cohutta	434	8	18.5
City Park	318	8	25.2	Tunnell Hill	240	13	54.2
Fort Hill Primary	534	17	31.8	Valley Point	1082	24	22.2
Fort Hill Jr. High	517	17	32.9	Ateooga	246	17	69.1
Emery St. (Negro)	328	3	9.1	Dawnville	461	9	19.5
				Varnell	241	4	16.6
Totals	3023	76	25.1		4557	139	30.5

* Attack rate per 1000.

Rural schools were somewhat more heavily involved than urban ones in terms of over-all rate, as indicated in Table 6. The magnitude of most of these attack rates is, in general, of the order of the rates pertaining to the age groups in primary schools; in the case of the high school, the attack rate approximates that for the 10-to-20-year age group in the general population. The low rate in the school for Negro children probably reflects primarily the low rate in the Negro population in general.

The cause of the rather wide variation in rates in the primary schools is not entirely

* Drake, M. E.; Barondess, J. A.; Bashe, W. J., Jr., and Stokes, J., Jr.: Unpublished data.

clear; it may be related to degrees of crowding, although this degree of variation would not have been expected on that basis.

It is of importance in delineating high-risk groups in this outbreak to note that 215 of the 335 cases in the epidemic occurred in school children; i. e., 64.3% of the cases occurred in this group. Many families were seeded with the virus by children who apstrued as additional evidence that the schools served as loci of dissemination of the disease.

Secondary Cases.—Secondary cases were defined, for the purposes of this study, as those with onsets from 14 to 50 days after the onset of the disease in another member of the same household. The criteria for diagnosis were the same as those outlined above. Since other studies † have shown that sur-

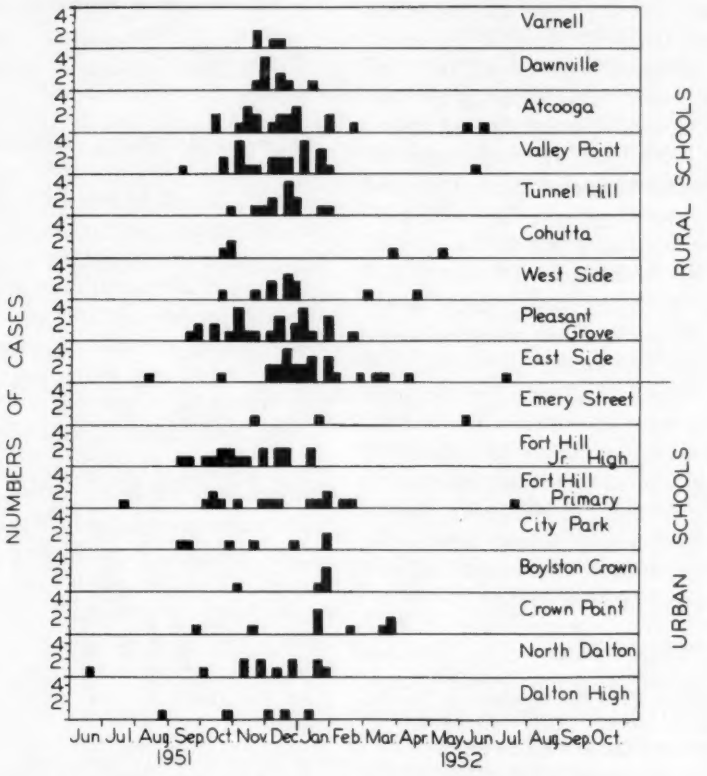

Fig. 2.—Epidemic hepatitis in Whitfield County schools.

parently contracted the disease from school contacts, secondary cases later occurring in the family unit. Thus, the contribution of school contacts to the epidemic may be considered to be even higher than the 64.3% figure derived above, in that some secondary cases were indirectly due to such contacts.

It will be noted in Figure 2 that the cases in the various schools tended to occur in the form of discrete outbreaks; this may be conveying family contacts of cases with appropriate liver-function tests may reveal many more cases than are apparent from clinical examination alone, it is probable that the secondary incidence in families noted in this epidemic is a minimal figure, and in all likelihood the true secondary attack rate is several times the rate obtained by use of our diagnostic criteria.

† Reference 25 and Knight, V., and Drake, M. E.: Unpublished data.

There were 27 diagnosed cases with onsets from 14 to 50 days after onset of the disease in another member of the same household, and these occurred in 18 families, of the total of 260 families involved in the outbreak. Thus, approximately 7% of the families affected in this epidemic had clinically-apparent secondary cases.

The total of family rosters of all cases in the epidemic was 1345. Subtracting the 308 primary cases, there remain 1037 persons exposed in the home. Of these, 27 developed clinically diagnosable hepatitis within 14 to 50 days after onset of the index case in the family; the secondary attack rate is, therefore, 26 per 1000. Thus, the risk of developing overt hepatitis after exposure in the home was at least twice as great as that in the general population, among which the over-all attack rate was 12.8 per 1000 (*v. s.*).

The mean interval between onset of the index case and onsets of secondary cases was 28.7 days, and the median interval was 28 days. Since patients with infectious hepatitis have been shown ‡ to excrete virus in their stools for several days before the appearance of jaundice, and may perhaps excrete it before the appearance of symptoms, the incubation period in this outbreak is probably somewhat longer than these figures indicate, i. e., of the order of 30 to 33 days.

‡ References 19 and 20.

TABLE 7.—*Attack Rates for Members of Families of Cases by Intervals After Onset of the Initial Case in the Family**

Interval After Onset of Initial Case in Family, Days	Subsequent Cases, No.	Attack Rate/1000 Exposed Persons
0- 5	19	18.3
6-10	15	14.7
11-15	10	9.9
16-20	5	5.0
21-25	3	3.0
26-30	7	7.1
31-35	2	2.0
36-40	4	4.1
41-50	4	4.1
50 & over	2	2.1
All cases	71	68.5

* Data based upon 1037 exposed persons of all ages.

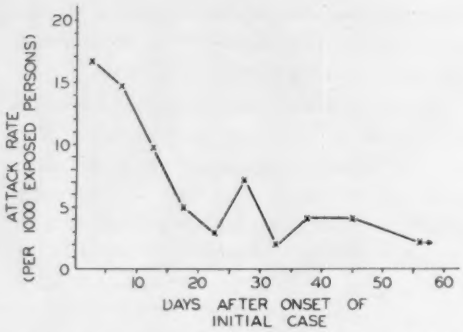

Fig. 3.—Attack rates for members of families of cases, by intervals after onset of the initial case in the family.

Table 7 and Figure 3 summarize the experience of families in which multiple cases developed. It will be noted that high attack-rates, reflecting coprimary cases, obtained in these families in the 10 days following the appearance of the initial cases and that these rates progressively fell off until the 26- to 30-day period was reached, when secondary cases appeared as a sharp wave and attack rates among the remaining susceptibles rose sharply. This interval, as indicated above, may be used as an approximation of the incubation period in this outbreak.

The epidemiological role of the family unit in this epidemic is emphasized by the fact that 18.5% of the 260 families involved in the outbreak had multiple cases. Most of these were either coprimary or secondary cases, only two occurring more than 50 days after the onset of the initial case in the family.

Water Supply Transmission.—There is no evidence to suggest that water supplies were involved in the spread of the disease. Many of the rural families had their own dug wells; furthermore, the city cases appeared without regard to water-distribution lines. In addition, the character of the epidemic curve suggests person-to-person transmission rather than common-source infection. Finally, bacteriologic examination of the city water has never indicated fecal contamination after treatment.

Milk Supply Transmission.—There is no evidence that sources or routes of distribu-

tion of milk were involved in the origin or spread of the epidemic. Attack rates on the basis of volume supplied did not differ significantly for the major milk companies in the area. Further, careful dairy inspection is maintained; all the milk consumed in the area is periodically evaluated in terms of U. S. Public Health Service criteria and is of high grade.

Insect and Animal Transmission.—All patients were queried with respect to insect and animal exposures, and most of the homes were inspected by an epidemiologist or nurse-epidemiologist. No evidence was obtained which would suggest that flies, mosquitoes, rats, mice, or other insects or animals might be incriminated in the spread of the epidemic. Most involved families had not noted particular contacts of this nature in the several months prior to appearance of the disease. In general, as mentioned above, animal and insect problems are not of great magnitude in Whitfield County.

General Sanitation.—Homes of patients involved in the outbreak were grossly graded as "poor," "fair," "good," or "excellent" on the basis of general cleanliness, sanitary facilities, and degree of crowding. Most of the cases came from homes graded "good" or "fair" on this basis; relatively few cases came from homes of the highest or lowest economic groups. In general, it appeared that the distribution of types of homes from which cases came approximated the distribution in the community at large.

Genesis of the Epidemic.—As mentioned above, infectious hepatitis has apparently shown low-grade endemicity in this region for some time. No clear-cut evidence was developed in the course of this investigation to suggest that the disease was imported from another area, although travel between Whitfield County and other parts of the state is frequent and hepatitis was epidemic in several areas in Georgia during the year prior to the outbreak. It is apparent that spread either from an endemic case or from another area might have resulted in the seeding of Whitfield County with the virus.

In connection with the former possibility, it was of considerable interest to find that the last known endemic case prior to the outbreak was related to the first case in the epidemic. C. M., a 30-year-old white male mechanic, was mildly ill in November, 1950, with anorexia, nausea, abdominal pain, and mild jaundice. The illness lasted one week; at no time did he feel sick enough to go to bed.

In June 1951, Mrs. E. P. (Case 1), a 34-year-old white woman who was the sister-in-law of C. M., developed severe hepatitis and died. Investigation revealed that opportunities for contact between these two persons had been numerous, since the families lived near each other and visited each other frequently.

When seen in October, 1951, 11 months after his illness, C. M. felt well but was found to have marked hepatosplenomegaly. Liver-function tests (serum bilirubin, cephalin-cholesterol flocculation, thymol turbidity, and flocculation [30]) gave normal results.

Because of the possibility that this man might be a fecal carrier of the virus, a 20% saline suspension of his stool was fed to each of six human volunteers. The latter were healthy young men in a penal institution, with no known history of jaundice and with normal physical examinations and liver-function tests. They were seen several times weekly by a physician, and batteries of liver-function tests were run at weekly intervals. None of these men developed hepatitis.

Several possibilities exist as explanations for this result: 1. In the chronic carrier state, fecal excretion of the virus may not necessarily be constant but, rather, may be intermittent. Thus, examination of a random stool specimen might give negative results despite the existence of a fecal carrier state. Such a phenomenon has been noted in other diseases in which fecal carriers are a problem, most notably in the case of typhoid fever. 2. The number of volunteers employed in this experiment may have been too small to provide cases of hepatitis if virus were present in low titer in the material fed them.

It may be estimated that with use of six susceptible volunteers and an infectious material capable of causing a 30% attack rate the chances of getting one case of hepatitis are about 88%. With material capable of causing a lower attack rate, the chances of getting a case in a volunteer are even lower. 3. The man may not be fecal carrier of the virus at all, whether or not his hepatosplenomegaly is related to his bout of infectious hepatitis. In the final analysis, his relation to the epidemic remains problematical.

Whatever its genesis, the epidemic appears to have spread through the community by person-to-person contact. Evidence for this includes the duration and shape of the epidemic curve, the widespread and centrifugal geographic distribution of the disease, the increased rate of spread in families of cases, and the fact that 99 of the total of 335 cases (29.6%) had had known contact with icteric cases before onset. Although this figure may be somewhat high, since many of the patients did not remember exactly when such contacts occurred and some of them may have been outside the known range of incubation periods of the disease, a considerable proportion of the cases appears to have had contact with other cases from two to seven weeks before onset.

Use of Gamma Globulin in the Epidemic.—During the course of this epidemic, 659 persons were injected intramuscularly with normal adult human gamma globulin (immune serum globulin) by the Dalton-Whitfield County Health Department; these people came to the Health Department voluntarily when it was made known that the material was available for those who desired it. In addition, a small number received the material from local physicians. The dose used was about 0.01 cc. per pound of body weight, which has been shown, as mentioned above, to afford to exposed susceptibles a high degree of protection against infectious hepatitis, when given more than five to seven days before the appearance of symptoms.

The age range among recipients of gamma globulin was 1 month to 69 years, with most of the persons being children or young adults. Although the exact ages of all persons injected were not known, ages of a representative sample, about half, were known; these data are summarized in Table 8. It will be seen that the group receiving gamma globulin was weighted with persons in the highest-risk age groups. In addition, many of those who came to the Health Department for gamma globulin did so because of known exposure to a case of hepatitis in the home. Thus, it may be considered that the risk of infection among these persons was even higher than that in corresponding age groups in the general population. One would expect that among the 659 persons who received gamma globulin several cases of hepatitis might occur, especially when it is realized that the time distribution of these injections correlated closely with the epidemic curve, so that opportunities for exposure to the virus in the injected group were comparable to, or even greater than, those among the general population.

TABLE 8.—*Age Distribution of Gamma Globulin Recipients Compared with General Population*

Age, Yr.	Gamma Globulin Recipients, %	General Population, %
<1	6	1.9
1-10	64	19.7
1-20	78	41.5

Among the entire group of 659 who received gamma globulin, there were 7 subsequent cases of infectious hepatitis. The over-all attack rate in this group was thus 10.6 per 1000. This figure is roughly comparable to the rate for the general population of the area, 12.8 per 1000. It is important to note, however, that of these seven cases, one developed on the same day the gamma globulin was given; two occurred 6 days after injection; two, on the 7th day; one, on the 16th day, and one, on the 21st day. The latter two cases must be classed as gamma globulin "failures," since the disease was not prevented from developing more than five to seven days after the material was administered; if the cases occurring on or prior to the 7th day are excluded, the

attack rate for the injected group becomes 3.1 per 1000, a figure striking lower than the rate for the general population.

The effect of gamma globulin in protecting exposed susceptibles may also be demonstrated if we consider only those persons who had been exposed to hepatitis in the home prior to receiving the material. There were 287 such persons in the injected group; within the 14- to 50-day period after appearance of the index case in the home, which we have used in defining secondary cases, no hepatitis developed among these 287 persons. Had the secondary attack rate in families, i. e. 26 per 1000, as developed above, applied to this group, 8 cases of hepatitis would have been expected, rather than none. This difference is highly significant statistically, when subjected to the χ-square method of analysis.

Thus, under field conditions, gamma globulin had a striking protective effect, even when administered to persons under a relatively high risk of infection.

COMMENT

We have presented data on an outbreak of infectious hepatitis which spread through a community by person-to-person contact over a period of 15 months, involving 335 persons in 260 families. Striking selection of the younger age groups was noted, and examination of appropriate attack rates indicated that the school and family groups were the chief loci of dissemination of the virus.

Gamma globulin has been shown to afford a high degree of protection of exposed susceptibles under field conditions, when administered in doses approximating 0.01 cc. per pound of body weight, more than seven days before the appearance of symptoms.

It would seem that, in future epidemics, efforts at control should proceed from these facts and that gamma globulin should be administered to persons in high-risk groups, namely, to school and family contacts of cases. It is quite likely that a significant degree of control could be achieved even if administration of the material were restricted to persons in these groups under the age of 20. Prior studies § have indicated that special attention should also be given to the protection of pregnant and postmenopausal women.

Further studies, in any event, should attempt to develop practical methods for control of epidemics of infectious hepatitis; the availability of a substance which is effective in protecting exposed susceptibles under field conditions and the magnitude of the problem raised by the frequency and prolonged courses of such epidemics, together with the frequently widespread and prolonged morbidity resulting from the disease, underline the importance of such studies, as pointed out earlier in this paper.

SUMMARY

An epidemic of infectious hepatitis is described, occurring in a combined urban and rural population and affecting chiefly the younger age groups. Spread appeared to have occurred by person-to-person contact; the incubation period in this outbreak approximated 30 to 33 days. A high secondary attack rate in families was noted, and over 64% of the cases occurred in school children.

Evidence of the prophylactic effectiveness of normal human gamma globulin under field conditions was obtained, and this material has been shown to reduce the secondary attack rate in families of cases.

The possibility that the outbreak might have originated in a chronic fecal carrier of the virus was considered and explored, although this person could not be associated definitely with the genesis of the epidemic.

The importance of studies oriented toward determining the most efficient and effective method of applying gamma globulin in epidemics of infectious hepatitis is emphasized; certain possibilities are briefly discussed.

Aid was given in these studies by the staff of the Dalton-Whitfield County Health Department, the members of the Whitfield County Medical Society, Officials of the Department of Institutions and Agencies of the State of New Jersey, Drs. Werner

§ References 49 and 50.

and Gertrude Henle, and Dr. A. D. Langmuir, of the Epidemiology Branch, Communicable Disease Center, U. S. Public Health Service.

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The Effect of Healed Subacute Bacterial Endocarditis on Cardiac Dynamics

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In essence, this report deals with the present status of 16 patients alive approximately 10 years after recovery from the active phase of subacute bacterial endocarditis. Ten of these patients were included in a report published in 1947.[1] In 1948 we presented to the Midwest Regional Meeting of the American College of Physicians the clinical status of 21 approximate 4-year survivors. Twelve of these were asymptomatic. Nine were definitely handicapped, ranging from house-confined invalidism to one or more episodes of congestive failure.

When we consider that 19 of these patients had been free of cardiac symptoms and able to lead unrestricted lives prior to the occurrence of bacterial endocarditis and that, of the other 2, only 1 had had an episode of congestive failure, it seemed reasonable to assume that the marked increase in the number with definite cardiac symptoms was due to the effect of the bacterial disease on the endocardium or myocardium or both. Study of the hearts of nine patients dying during therapy [2] confirmed the impression that the residual effect of bacterial endocarditis on valves and myocardium was uniformly more severe than that of an initial attack of rheumatic fever.

Eight of the 19 previously asymptomatic patients had x-ray and/or electrocardiographic evidence of cardiac abnormality at the time of admission for therapy of active endocarditis. X-ray abnormalities consisted of 14% to 43% increase in transverse diameter, as seen in the P-A projection, or occasional differential chamber enlargement. Electrocardiographic abnormalities were chiefly minimal and nonspecific. There was one instance each of P mitralis, first degree A-V block, and bundle-branch-system block. Neither of the two patients having previous cardiac symptoms showed roentgen or electrocardiographic abnormalities on admission.

Between the 4th and 10th year of survival 5 of the 21 patients died.

The first patient died during an attack of acute rheumatic fever in the fourth year of recovery from bacterial endocarditis. The original murmur was that of mitral regurgitation. He had a second attack two years after the first. At this time additional murmur of aortic stenosis was present. Except for this he was able to work full time as a chemist, without cardiac symptoms, until the fatal attack of rheumatic fever. Whatever additive cardiac damage was caused by the two attacks of bacterial endocarditis, it was not sufficient to cause significant disability, at least for four years.

The second, the house-confined cardiac invalid, developed signs of chronic pyelonephritis during the first year of survival, with resultant progressively increasing hypertension. Valvular lesion was mitral stenosis and

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regurgitation. Death, in the seventh year, was due to acute myocardial infarction secondary to coronary occlusion by a piece of calcium dislodged from a healed mitral vegetation. This patient's disability certainly resulted from her bacterial endocarditis. She had a second attack six months after recovery from the first. The severe hypertension must have been a factor. This is the only instance of chronic pyelonephritis we have encountered in patients recovering from bacterial endocarditis.

The third death was due to carcinoma of the prostate in the eighth year. This man was 55 at the time of active bacterial endocarditis, and was one of the two having previous cardiac symptoms. Valve lesion was mitral regurgitation. Transverse cardiac diameter was increased 32%. During the fourth year a mitral diastolic murmur was noted and the transverse cardiac diameter was 46% increased. Marked shortness of breath on exertion was noted, and maintenance digitalis was required. At first thought it might seem that this man's condition, subsequent to 1945, was the result of the bacterial endocarditis. But how many patients of age 55 with 32% cardiac enlargement and symptoms of cardiac insufficiency due to mitral regurgitation would fare any better over a four-year period? Judged on the basis of postmortem data from other patients, the healed mitral vegetations could well be responsible for the mitral diastolic murmur. But, as will be noted later, this does not necessarily mean a dynamically significant stenosis.

The fourth death occurred at the end of the ninth year. The patient, a woman aged 29 at the time, completed therapy in August, 1944. At this time x-ray showed the heart to be of normal size, although the electrocardiogram showed evidence of left ventricular enlargement. A loud apical systolic murmur was present, as well as a soft systolic and diastolic murmur along the left sternal border. She returned to full-time clerical work and an active social life. In 1948 the heart was 12% enlarged by x-ray. The electrocardiogram was unchanged. At that time she had had no subjective symptoms of cardiac incapacity. There were presystolic and systolic murmurs at the apex and a diastolic murmur over the aortic area. In 1950 the heart was 20% enlarged by x-ray, with left atrium and right ventricle predominating, and the clinical picture was unchanged. In July, 1953, the patient died, after an episode of severe gastroenteritis of four days' duration. Autopsy revealed severe myocardial scarring, moderate mitral stenosis and insufficiency, and calcification of the aortic valve with the cusps remaining functionally intact.

The condition of this patient's mitral valve, as well as that of the second patient above and two others not included in this series, was interesting. All were markedly deformed by calcific and fibrous nodules along the margins. The body of the leaflets, while thickened, was pliable, and the commissures not markedly agglutinated. The valve opening was 2.5 cm. Today we know that an opening of this size will not cause clinically significant impairment of cardiac dynamics. The valve opening in the three other cases was similarly adequate, suggesting that stenotic murmurs, developing after recovery from bacterial endocarditis, do not necessarily mean progressive disease or stenosing of the orifice. Likewise, when stenotic murmurs are present prior to bacterial endocarditis, subsequent increase in intensity and duration do not necessarily mean dynamically significant increase in stenosis as a result of bacterial endocarditis. The histology of the valves in this last case is interesting. In addition to severe scarring and calcification, the mitral and aortic valves showed scattered areas of perivascular infiltration with lymphocytes and plasma cells. Many fibroblasts were present. At one angle of the mitral valve were two small collections of fibrin containing chiefly plasma cells and overlying areas denuded of endothelium. Is it possible for this to occur periodically in connection with simple upper respiratory infections, or even spontaneously, and, on organization, seal the valves together? If this situ-

ation is repeated often enough, steadily increasing stenosis could develop without subclinical rheumatic fever being necessary. The patient never showed signs of active rheumatic fever during the nine years, and, in the opinion of our pathology department, the microscopic findings were not typical of rheumatic activity. No Aschoff bodies were found. The record is not clear as to involvement of the aortic valve by the original rheumatic fever. During active bacterial endocarditis there was also a difference of opinion regarding involvement of the aortic valve. During the nine subsequent years there were intermittent periods of definite ausculatory and vascular signs of aortic regurgitation. With the heart in the "dead" state the valve leaflets, while deformed by healed verrucae, were closed. The episodes of regurgitation were usually associated with or followed acute upper respiratory infections, suggesting transitory involvement of the valve which prevented complete apposition of the leaflets. During the brief final illness there were no signs of aortic regurgitation. We have observed transitory aortic regurgitation in one other patient still living. The severe myocardial scarring was a surprise. Here again, in the opinion of pathologists, it exceeded that which might be expected as a residual from the original rheumatic deformity. The important clinical point is that in spite of severe myocardial and valvular damage this patient's heart did not enlarge significantly and whatever impairment of cardiac dynamics existed it was not rapidly progressive and did not preclude an active useful life. The information gained from this patient is important in understanding the present status of most of the survivors under consideration in this report.

The fifth death occurred in the ninth year of survival. A woman aged 54 at time of endocarditis was previously asymptomatic except for a presumed episode of congestive failure 10 years previously. Therapy was completed in July, 1945. She continued to work as a hospital comptroller until four months before her death. In 1948 the heart size by x-ray was normal; auscultation revealed evidence of mitral regurgitation. From 1950 to her death, in December, 1953, she had several episodes of mild cardiac insufficiency and was easily fatigued and short of breath on mild exertion. Death was due to primary carcinoma of the liver. In 1949 she developed signs of arteriosclerotic heart disease with bundle-branch-system block and became moderately hypertensive. Cardiac size by x-ray did not increase. During the last two years she showed evidence of mild arteriosclerotic cerebral deterioration. Here again it cannot be said that additive cardiac damage incident to bacterial endocarditis was the cause of the decrease in cardiac efficiency during the last three years of life. The clinical course was that of arteriosclerotic cardiovascular disease.

In summary, it is our opinion that in only one of these four-to-nine-year survivors could a downhill course and death be attributed to residual damage of bacterial endocarditis. Unless carefully evaluated, inconsistencies in the subsequent clinical course of patients with similar or dissimilar evidence of cardiac damage prior to bacterial endocarditis may lead to unwarranted pessimism with reference to the over-all seriousness of the disease for those who survive. Detailed analysis of three of our living patients illustrates this. The influence of advanced age, hypertension, and arteriosclerotic heart disease is eliminated, the age range at time of bacterial endocarditis being 21 to 26. For purposes of identification they will be referred to as follows:

CASE 1.—Woman, age 24, mitral stenosis and regurgitation, asymptomatic prior to endocarditis. Duration of endocarditis before therapy, six weeks. Normal electrocardiogram and x-ray on admission in 1944.

CASE 2.—Man, age 21, mitral regurgitation, previously asymptomatic, with normal x-ray and electrocardiogram on admission in 1944. Duration of endocarditis before therapy, 15 weeks.

CASE 3.—Man, age 26, numerous attacks of rheumatic fever and pericarditis since age 6, mitral and aortic stenosis and regurgitation, cardiac outline 48% increased, aortic configuration, electrocardiogram normal on admission in 1945. Duration of

endocarditis before therapy, 15 weeks. No previous episodes of congestive failure, but living a sedentary life.

During the active phases of the disease, Cases 1 and 2 showed minimal electrocardiographic abnormalities which were not permanent. Case 3 was unchanged. At time of discharge Case 1 showed 48% increase in transverse cardiac diameter by x-ray, with a contour thought to be chiefly that of mitral regurgitation. X-ray of Case 2 showed no change. Case 1 did well clinically for 18 months, then went into failure and has followed a progressively downhill course. Auricular fibrillation became established during the fifth year. Transverse diameter of the heart by x-ray had not increased at this time, but the contour showed the effects of mitral stenosis. There have been seven episodes of failure. Today she is barely maintained at almost house-confined activity by continuous use of digitalis, mercurials, diet, and resins. X-ray shows 76% increase in transverse diameter with marked enlargement of all chambers, except right atrium, but with left atrium and right ventricle predominating. Electrocardiogram shows left and right ventricular hypertrophy with left strain pattern.

Case 2 was considered our best clinical result in the four-year report. A mitral diastolic murmur appeared six months after cure of his endocarditis. At five years his heart was 15% enlarged; he was asymptomatic on an unrestricted level and working daily as an executive. Six years after recovery he had sudden onset of auricular fibrillation and congestive failure. The heart was 10% enlarged and of mitral stenotic contour. Today he is still working steadily and has no complaints, but he gasps and breathes heavily at rest. Maintenance doses of digitalis are required. There have been no more episodes of acute failure. X-ray shows 37% increase in cardiac size, predominantly mitral stenotic with little or no hypertrophy of the left ventricle. Electrocardiogram shows right ventricular hypertrophy but no strain pattern. For a period of two and a half years prior to onset of fibrillation, murmurs of aortic stenosis and regurgitation were present, with peripheral signs of regurgitation. These disappeared three months after onset of fibrillation.

Cardiac catheterization in 1954 showed almost the same degree of pulmonary artery and right ventricular hypertension in both Case 1 and Case 2. Resting cardiac index was low, 1.7 and 2.0, respectively. It did not increase significantly on exercise. A-V difference was above normal at rest and increased markedly on exercise. Judged by catheterization data, these two hearts are almost equally impaired; yet clinically (including x-ray and electrocardiogram) there is a marked difference. Discrepancy between catheterization data and what the patient can do is a common finding. It must be remembered that the exercise used does not represent much in terms of activity for daily living.

Why did these two patients do well, one for 18 months and the other for 6 years, then suddenly go into failure? Could a progressively increasing stenosis be the major factor? How much of a factor was the residual myocardial damage? Why should there be practically no difference in catheterization findings yet considerable difference in x-ray findings and marked difference in physical ability?

The marked enlargement of the heart during the active endocardial infection in Case 1 suggests much more severe myocarditis than occurred in Case 2. We believe that this, rather than increasing mitral stenosis, explains the early failure and subsequent course in Case 1. Judged by the clinical course and roentgen findings, Case 2 followed the pattern of gradually increasing mitral stenosis, such as is seen in rheumatic mitral disease uncomplicated by bacterial endocarditis. On the basis of what we have observed from postmortem studies, we believe that if the mitral stenosis in Case 2 were simply the result of healing of the bacterial endocarditis the diastolic murmur should have been present before the patient left the hospital and the stenosis should not increase in severity. If this hypothesis is correct, the present status of Case 1 is the result of the effect of

bacterial endocarditis on the myocardium, rather than on the valve, while that of Case 2 is the result of the effect on the valve of the underlying rheumatic process.

Case 3 required 14½ weeks of intensive therapy. The course was stormy. Electrocardiogram showed no abnormalities during active phase. Not gainfully employed for two years after recovery. Frequent acute pericarditis and upper respiratory infections. In severely incapacitated group in four-year study. Gradual clinical improvement in cardiac efficiency began early in fifth year and has continued. No further pericarditis. Working as a meter reader, requiring much stair climbing, past four years. Now asymptomatic. X-ray today shows 48% increase in size of heart, with marked enlargement of left and right ventricles and some of the left atrium, without enlargement of pulmonary root vessels. Cardiac catheterization showed slight pulmonary and right ventricular hypertension, with normal A-V difference at rest, increasing only slightly on exercise. Normal cardiac index at rest, increasing only 50% on exercise, while the oxygen consumption more than doubled. Although the response of the cardiac index to effort was below normal, there was a good overshoot on Valsava maneuver. The hydrodynamically significant lesion was considered to be aortic regurgitation with slight inadequacy of the left ventricle. Electrocardiogram shows left ventricular hypertrophy and early strain pattern and some right ventricular hypertrophy. Today the mitral murmurs are not present. They disappeared at about the time clinical improvement began. Several examiners confirmed their previous presence and present absence. The only explanation we can offer is that similar behavior has been observed in connection with acute rheumatic fever.*

In spite of a bad history, objective evidence of at least moderately severe cardiac involvement at time of endocarditis, a long stormy therapeutic course followed by five years of repeated attacks of pericarditis (presumably rheumatic), and marked clinical diminution in cardiac reserve, this patient did an about-face and today is clinically better than either in Case 1 or Case 2. Both x-ray and catheterization findings in this case are in line with the present clinical status. On the basis of the evidence today, it would appear that the mitral valve was not involved in the bacterial endocarditis and that the additive deformity of the aortic valve was not severe. The fact that the heart size did not increase during the stage of active bacterial endocarditis (it actually decreased slightly), and that today the transverse diameter is 6% less than it was 10 years ago, supports the contention that myocardial damage by the bacterial endocarditis was not severe. This case further emphasizes the possible error in attributing clinical evidence of severe cardiac damage to residuals of bacterial endocarditis rather than to a continuing rheumatic process.

Examination of the present status of the 16 patients alive approximately 10 years after recovery from bacterial endocarditis, with reference to the presence or absence of evidence of progressive cardiac incapacity, reveals that they can be placed into three groups. Cardiac catheterization has been done on representatives of each group.

The criteria we have selected for grouping these patients are as follows:

Group I
1. American Heart Association functional Class I
2. Cardiac enlargement 10% or less (by x-ray), or no increase in heart size in 10 years

Group II
1. American Heart Association functional Class II
2. Cardiac enlargement (by x-ray) 10%-20%

Group III
1. Dead of heart disease
2. Below American Heart Association functional Class II
3. Cardiac enlargement 20% or more

It must be pointed out that these three groups may be artificial, since the criteria for establishing them may be arbitrary. It should also be pointed out that the number of patients included in this study is too small to permit statistical analysis. For this reason, we avoid using percentages but show the total range of variation of the several phenomena we have studied.

When these criteria are applied to the 16 living patients included in this study, 11 fall into Group I, 2 into Group II, and 3 into Group III.

When these groups are studied from the standpoint of possible prognostic information, interesting data come to light (Table).

* Reference 3 and Luisada, A.: Personal communication to the authors.

It is to be noted that absolutely no correlation exists which might lend prognostic significance to any of the above data. The duration of active infection before therapy has been considered for some time to be of great importance in determining the amount of valvular and myocardial damage and thus influencing the long-term prognosis of these patients. It is apparent that this has not been true in this series. The same can be said of the duration of therapy necessary to arrest the disease.

Cardiac catheterization was performed on five patients in Group I. In two the cardiac output at rest and on exercise was normal. X-ray and electrocardiogram were normal. One was found to have a small interventricular septal defect instead of mitral regurgitation. The other has borne three children, in addition to leading a very active life, without symptoms. The third is Case 3, previously discussed. The fourth showed mitral regurgitation not significantly affecting cardiac dynamics. A slight reduction in cardiac index at rest with inadequate increase on exercise was interpreted as resulting from mild chronic coronary insufficiency. The fifth showed slight impairment of hemodynamics due to mitral regurgitation. X-ray and electrocardiogram are normal. She has been continuously asymptomatic on an unrestricted level.

The one patient in Group II subjected to catheterization showed only slight impairment of hemodynamics due to mitral regurgitation. She leads an unrestricted life and has borne three children. The transverse cardiac diameter was 25% increased at time of bacterial endocarditis and has not increased. Electrocardiogram is normal. She was put in Group II because of pulmonary hypertension on exercise and definite increase in size of left atrium and left ventricle by x-ray. We believe that her present condition is not significantly different from that of the average case of mitral regurgitation presenting a 25% enlargement of the heart nine years ago. The other patient in Group II was 52 at time of bacterial endocarditis in February, 1945. The murmur was that of mitral regurgitation. He had previously had a myocardial infarction. He has worked steadily as an otolaryngologist without symptoms. X-ray shows 20% increase in cardiac size. Little, if any, cardiac damage can be attributed to bacterial endocarditis.

Data on Sixteen Patients with Healed Subacute Bacterial Endocarditis

	Group I (11 Cases)	Group II (2 Cases)	Group III (3 Cases)
Range of age (on admission), yr	9-74	19-52	21-34
Organism (S. viridans or other)	Viridans 0 Other 1	Viridans 2 Other 0	Viridans 2 Other 1
Range of sensitivity to penicillin *	0.06-6.0 (1 not sens.)†	0.02-6.0	0.04-6.0
Duration before therapy, wk	7-29	2-39	15-20
Duration of therapy, wk	8-23	4-16	6-18
Murmurs changed in 10 yr	1 case	1 case	1 case

* This lack of correlation we predicted in 1944-1945.
† H. parainfluenzae. Cure obtained with combined penicillin and sulfonamide therapy.

All the patients in Group III were catheterized. Two have already been discussed as Cases 1 and 2. The third patient has Marfan's syndrome. The present marked diffuse cardiac enlargement and original dilatation of the aorta, which has now progressed to an aneurysm of the base and ascending portion, are due to the cardiovascular lesions of that disease and not to bacterial endocarditis. Up to one year ago he was put in Group II.

CONCLUSIONS

On the basis of this study of 16 patients who are living approximately 10 years after recovery from bacterial endocarditis the following points can be brought out:

1. Bacterial endocarditis adequately treated does not appear to be as damaging to the heart as was previously believed.[4]

2. The ultimate fate of the large majority of patients cured of bacterial endocarditis is determined not by the residuals of the bacterial process but by the severity or nature of the preexisting cardiac damage or anomaly and the subsequent presence or absence of rheumatic activity, coronary artery disease, and hypertension.

3. Severity of the myocarditis incident to the active phase of bacterial endocarditis plays a greater part in determining future cardiac impairment than does residual valve damage.

4. The duration of activity of the infectious process prior to therapy does not seem to influence the future prognosis.

5. Careful roentgen study in four projections plus careful clinical evaluation gives practically as much information concerning the severity of preexisting and subsequent cardiac damage as does cardiac catheterization.

6. Cardiac catheterization is necessary when there is a question as to the exact anatomic diagnosis, or when the severity of subjective symptoms is out of proportion to the physical, roentgen, and electrocardiographic findings.

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Nitrofurantoin

Clinical and Laboratory Evaluation

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The purpose of this report is to present laboratory and clinical studies with nitrofurantoin. This drug is a derivative of the nitrofurans and has the chemical formula N-(5-nitro-2-furfurylidene)-1-aminohydantoin. It was synthesized and shown to have antimicrobial activity by the Eaton Laboratories in 1952. Norfleet and others have shown that nitrofurantoin is particularly effective in urinary tract infections due to Proteus, that it is well tolerated, and that it does not have a marked effect on the bacterial flora of the bowel.* Because infections with Proteus, toleration of antimicrobial drugs, and the development of infections due to the derangement of bowel flora by antibiotics are three of the most pressing problems in large general hospitals, we felt that further investigation of nitrofurantoin was warranted.† Forty per cent of nitrofurantoin appears in the urine within six hours after it is administered by mouth; therefore, this study, as have been previous ones, is concerned with the use of nitrofurantoin in the treatment of urinary tract infections.‡

In vitro, the type of resistance to nitrofurantoin exhibited by bacteria, the mode of action of nitrofurantoin, and the "antimicrobial spectrum" of nitrofurantoin were studied. In addition, 64 hospital patients were given nitrofurantoin. Toleration, toxicity, effectiveness, and the emergence of new species of bacteria during therapy were studied. Four cases were selected for brief presentation as examples of our over-all experiences.

IN VITRO STUDIES

I. Type of Resistance

One of the best-studied means by which bacteria resist drugs is by the survival of resistant mutants that arise spontaneously within the infection.[10] There are three types of mutants: those that are slightly more resistant than the parent strain, those that are moderately more resistant than the parent strain, and those that are markedly more resistant than the parent strain. The slightly more resistant mutants have been named the "penicillin type," because they occur in vitro in penicillin-sensitive strains of Micrococcus pyogenes.§ The markedly more resistant type of mutants are called the "streptomycin type," because they occur in cultures of almost all bacteria that are sensitive to streptomycin.[10]

The first experiment was designed to determine the kind and frequency of mutants resistant to nitrofurantoin.

Material and Methods.—A strain of Esherichia coli isolated from the urine of a patient was used as the test organism. It was inhibited by 0.38γ/ml. of streptomycin and 6γ/ml. of nitrofurantoin. One milliliter aliquots of an 18-hour growth in dextrose broth (3×10^8 bacteria) were placed on Petri dishes. Eight milliliters of nutrient agar and 1 ml. of distilled water that contained increasing concentrations of streptomycin and nitrofurantoin then were added to the individual Petri dishes. The number of colonies that grew on each plate in 48 hours was counted. Subcultures of individual colonies were made and tested for their sensitivity to streptomycin and nitrofurantoin.

Results.—Table 1 shows the comparison between the type of resistance exhibited by

The trade name for nitrofurantoin is Furadantin, Eaton Laboratories, Inc., Norwich, N. Y.

From the infectious disease control unit of the department of medicine of the Milwaukee County General Hospital and Marquette University School of Medicine.

* References 1-6.

† References 7-9.

‡ References 2, 4, and 6.

§ References 10 and 11.

TABLE 1.—*Resistance of E. Coli* to Increasing Concentrations of Nitrofurantoin and Streptomycin*

Concentration Drug, γ/Ml.	Number of Colonies Growing in Plates Containing Nitrofurantoin	Number of Colonies Growing in Plates Containing Streptomycin
2.5	Overgrown	48
5	Overgrown	73
10	Overgrown	39
20	0	40
40	0	9
400	0	0

* The parent strain of E. coli was inhibited by 0.38 γ/ml. of streptomycin and 6 γ/ml. of nitrofurantoin. Each aliquot contained 5×10^7 bacteria.

the strain of E. coli to nitrofurantoin and to streptomycin. In the case of nitrofurantoin, no colonies grew that were significantly more resistant than the parent strain. In the case of streptomycin, resistant mutants appeared that were many times more resistant than the parent colony. A subculture from the plate containing the highest concentration of nitrofurantoin that allowed growth had the same sensitivity to nitrofurantoin as the parent colony. A subculture from the streptomycin plate that contained 40γ/ml. of this drug yielded a strain of E. coli many times more resistant to streptomycin than the parent strain (Table 2).

Another method by which bacteria resist drugs is by the production of a substance that destroys or inhibits the action of the drugs.[11] In the second experiment an indirect method was used to determine whether

TABLE 2.—*Sensitivities of Cultures of E. Coli Isolated from the Highest Concentrations of Nitrofurantoin and Streptomycin in Which They Could Grow*

Inhibitory Concentration of Drug, γ/Ml.	Streptomycin*	Nitrofurantoin*
0.38	OS	..
0.75	..	..
1.5	..	..
3	..	..
6	..	OS NS
12	..	..
25	..	..
50	..	..
100	..	..
>100	SS	..

* OS, original strain; SS, strain isolated from a plate containing 40 γ/ml. of streptomycin; NS, strain isolated from a plate containing 10 γ/ml. of nitrofurantoin.

strains of bacteria that were relatively resistant to nitrofurantoin produced a substance similar to penicillinase. It has been found that when resistance to an antimicrobial drug is mediated through an inhibiting substance the resistance of the organism increases with the strength of the inoculum.[11] For instance, 100 penicillinase-producing M. pyogenes organisms might be inhibited by less than 1γ per milliliter of penicillin, whereas 10,000,000 bacteria of the same strain would grow well in 1000γ per milliliter of penicillin. On the other hand, a streptomycin-resistant mutant of the same strain of M. pyogenes would grow in 1000γ per milliliter of streptomycin whether the inoculum was 100 bacteria or 10,000,000 bacteria.[11]

TABLE 3.—*Relationship Between Size of Inoculum and Resistance of a Strain of Proteus to Nitrofurantoin*

Number of Bacteria Used in Tube-Dilution Test [12]	Inhibitory Concentration of Nitrofurantoin, γ/Ml.
3×10^1	1.5
3×10^2	12
3×10^3	25
3×10^4	25
3×10^5	100
3×10^6	100
3×10^7	100

Material and Methods.—Strains of Proteus that were relatively resistant to nitrofurantoin were used. The sensitivities to nitrofurantoin of inocula of increasing numbers of bacteria were determined by the tube-dilution method. Our assumption was that, if an inhibiting substance was being produced, an increase in the inoculum would increase the resistance of the organism.[11]

Results.—In each of several experiments, the sensitivity to nitrofurantoin of the organisms appeared to be directly related to the inoculum used in the tube-dilution test (Table 3). This suggests that resistance to nitrofurantoin may be mediated through an inhibiting substance.‖

II. MODE OF ACTION

Two approaches were used to determine whether nitrofurantoin primarily was bacteriostatic or bactericidal.

‖ References 11 and 12.

First, the bactericidal modification of the tube-dilution test was done on a large series of sensitivity determinations.[12]

Method.—A 0.1 ml. aliquot was taken from each of the tubes in which the growth of the bacteria had been inhibited in the routine tube-dilution method of determining bacterial sensitivity to antibiotics. These aliquots were placed in 10 ml. of dextrose broth and incubated at 37 C for 48 hours. If the drug was bactericidal, no growth was noted. If only a bacteriostatic effect was achieved, growth was noted.

Results.—In almost every instance each clear tube, in the tube-dilution test, was found to be sterile. This indicates that nitrofurantoin had a killing effect and is in contradistinction to the results when the same test is done with the tetracyclines or chloramphenicol.

TABLE 4.—*Bactericidal Activity of Chlortetracycline and Nitrofurantoin Added to Flasks Containing an Eighteen-Hour Growth of E. Coli*

Time After Drug Was Added	Plate Counts from Chlortetracycline Flask*	Plate Counts from Nitrofurantoin Flask*
0	5×10^8	5×10^8
15 min.	2×10^7	1.4×10^5
3 hr.	2.8×10^7	6×10^3
6 hr.	5×10^8	No growth
24 hr.	No growth	No growth

* The concentration of drug in each flask was five times the minimal inhibitory concentration as determined by the tube-dilution method.

The bactericidal activity of nitrofurantoin then was compared specifically with that of chlortetracycline, which is known to be primarily bacteriostatic in action.[13]

Materials and Methods.—Strains of E. coli were planted in dextrose broth in two 250 ml. Erlenmeyer flasks and incubated at 37 C for 18 hours. Chlortetracycline and nitrofurantoin then were added to make a concentration five times greater than that necessary to inhibit the growth of the strain of E. coli. The flasks again were incubated at 37 C. One-milliliter aliquots were taken from each flask at 15 minutes, 3 hours, 6 hours, and 24 hours, and plate counts were made.

Results.—Table 4 shows a typical experiment in which nitrofurantoin was markedly more bactericidal than chlortetracycline. Although both were ultimately bactericidal in the concentration used, the nitrofurantoin began to kill the bacteria within 15 minutes and sterilized the cultures in 6 hours, whereas the chlortetracycline had no appreciable bactericidal effect within the same 6-hour period.

III. Comparative Activity of Nitrofurantoin and the Antibiotics Against Five Species of Bacteria (Fifty Strains)

Material and Methods.—Ten strains each of E. coli, Proteus, Aerobacter aerogenes, Pseudomonas aeruginosa, and M. pyogenes were tested for their bacteriostatic sensitivity to nitrofurantoin, chlortetracycline, oxytetracycline, tetracycline, chloramphenicol, and neomycin. The tube-dilution method was used.[12] Nitrofurantoin was dissolved in polyethylene glycol, and this solution then was dissolved in distilled water. The minute amount of polyethylene glycol in the final dilutions was shown to have no antimicrobial activity.

Results.—Table 5 shows the sensitivities of the 50 strains of bacteria to nitrofurantoin and the antibiotics. On a weight-for-weight basis, nitrofurantoin has about the same activity as the antibiotics. However, this weight-for-weight comparison must be viewed with the realization that the concentration of nitrofurantoin reaches levels of 40 mg. per 100 ml., whereas the antibiotics reach levels in the urine of 100γ per 100 ml.¶ The two species that appear most sensitive to nitrofurantoin are M. pyogenes and E. coli. Seven of the 10 strains of Proteus were inhibited by the concentrations of nitrofurantoin that were used. The spectrum of nitrofurantoin is most similar to that of chloramphenicol. The activity of nitrofurantoin against M. pyogenes and Proteus particularly is noteworthy because these two species of bacteria now cause the majority of our problem infections.# The resistance of P. aeruginosa to nitrofurantoin has been noted by other investigators and proved by published clinical experiences.* The activity of the antibiotics against the Gram-negative bacilli is similar to that recorded in a previous report.[15]

¶ References 3 and 14.

References 7 and 9.

* References 2-4.

TABLE 5.—*Comparative Activity of Nitrofurantoin and Antibiotics Against Five Species of Bacteria* *

Concentration, γ/Ml.	Chlortetracycline	Chloramphenicol	Oxytetracycline	Streptomycin	Tetracycline	Neomycin	Nitrofurantoin
<0.38	S3, S4, S5	.....	C8, C9, C10 S2, S3, S4 S5, S7	S5, S7	C1 S4	C8, C9, C10 S2, S3, S4 S5, S6, S7	S4, S7
0.38	C1, C6, C7 S10	.....	C1	S9	C5 S5, S7	C1, C2, C5 S8, S9	S9
0.75	C5, C10 S7	.....	C5 S10	.....	.....	S10	.....
1.5	.....	C1, C5	C6, C7 Ps2, Ps4, Ps10	.....	C4, C10	C3 A5	S10
3	C9 Ps2 A1, A7	C4, C6, C7, C10 A7 S9	Ps5, Ps7, Ps9	C1, C9, C10 P8 S8	C6, C7, C8 Ps2, Ps3 S10	C4, C6, C7 Ps2, Ps3 P8, P9 A6	C1, C5
6	C4 Ps5, Ps7, Ps9 A5 S6	P7 A8 S5, S6, S7 S8, S10	C4 Ps3 A1, A3, A5, A8	C8 Ps2, Ps4 P10 S3, S4	C9 Ps5, Ps7, Ps9 Ps10 P8 A3	Ps5, Ps7, Ps9 Ps10 P5, P7 A2	C4, C6, C7 C8, C10 A8 S6
12	C8 Ps4, Ps10 A3, A8, A9 S2	C8 Ps2 P3, P5, P6, P10 A3, A4	C2 S1	C6, C7 Ps3 P3, P4, P7, P9 A3 S6	Ps4 S6	Ps2, Ps6, Ps8 P4, P6, P10 A1, A3, A7, A8 A9 S1	C9 Ps4 S1, S2, S3, S8
25	Ps3	C9 P8, P9 A5, A6 S2, S4	P8 A7 S6	P2, P5, P6 A7 S10	A5, A8	P1, P2, P3	P5, P7, P8 A7
50	Ps6, Ps8 P6, P8, P9 A2, A4	C2 Ps1 P1, P2, P4 A9 S1	Ps6, Ps8	.....	C2 Ps6, Ps8 P6, P7 A2, A9 S2	.....	C2 Ps1 P3, P4, P6, P9 A5, A6, A9 S5
100	P4, P5, P7, P10 S1, S8, S9	C3 Ps7 A2 M3	P6 A4, A10	S2	C3 Ps1 P3, P4, P5, P9 P10 A7 S3, S8	A4	C3 P10 A2, A3
>100	C2, C3 Ps1 P1, P2, P3 A6, A10	Ps3, Ps4, Ps5 Ps6, Ps8, Ps9 Ps10 A1, A10	C3 Ps1 P1, P2, P3 ,P4 P5, P7, P9, P10 A2, A6, A9 S8, S9	C2, C3, C4 Ps1, Ps5, Ps6 Ps7, Ps8, Ps9 Ps10 P1 A1, A2, A4, A5 A6, A8, A9, A10 S1	A4, A6, A10 S1, S9	A10	Ps2, Ps3, Ps5 Ps6, Ps7, Ps8 Ps9, Ps10 P1, P2 A1, A4, A10

* C, Escherichia coli; Ps, Pseudomonas aeruginosa; A, Aerobacter aerogenes; S, Micrococcus pyogenes; P, Proteus.

CLINICAL STUDIES

METHOD

Sixty-four patients on the wards of the Milwaukee County General Hospital were given nitrofurantoin. Cases were chosen on the basis of there being an indication for treatment of their urinary tract infection. No attempt was made to exclude patients in whom complicating factors were present. This explains the relatively large number of patients in whom the results were equivocal. Sixty patients had urinary tract infections, and four patients had neoplastic or systemic lesions for which nitrofurantoin was used as a last resort. The age, race, sex, dosage used, duration of therapies, and diagnosis of the 60 patients with urinary tract infections are presented in Table 6. Previous medication; toleration of nitrofurantoin; effects on urine cultures, urinalysis, and body chemistry studies, and evidences of toxicity were noted insofar as was possible in each case. Interpretation of the final result was based on the opinion of the physician in charge of the patient and on the objective evidence

TABLE 6.—*Age, Sex, Race, Dosage, Duration of Therapy, and Diagnosis of Sixty Patients Treated with Nitrofurantoin*

	Patients, No.
Age, yr.	
20-40	7
41-60	12
Over 61	41
Sex	
Male	31
Female	29
Race	
White	47
Negro	13
Dosage, mg./day	
150	5
200	5
300	21
400	27
600	2
Duration of therapy, days	
0-3	9
4-7	27
8-11	7
12-15	8
16-19	3
>19	6
Diagnosis	
Pyelonephritis	32
Cystitis	27
Polycystic disease of kidney	1

in the hospital chart. Results were considered equivocal unless objective evidence of a therapeutic effect on the temperature curve, urine culture, or urinalysis was definitely recorded on the chart. Thus, in some cases the clinician was satisfied with the results but treatment was judged equivocal owing to lack of objective evidence of response.

Therapy was considered satisfactory if in addition to the subjective opinion of the clinician there was objective evidence of either eradication of the causative organism, a marked lowering of fever, or a definite improvement in the microscopic findings in the urine. Our experience in the treatment of chronic urinary tract infections has led us to believe that definitive cures of the infections are indeed rare. Therefore, we considered the treatment successful if the patient, when judged by all the standards we could study, had benefited from therapy with nitrofurantoin. Therapy was considered a failure if the patient did not improve or if the patient got worse during therapy.

RESULTS

Toleration of Medication: Nine of the 60 patients had difficulty in tolerating nitrofurantoin, and in each case the complaints were of nausea and some vomiting. However, in only one case was it necessary to discontinue treatment, and in three instances therapy was successful in spite of the nausea and vomiting. Table 7 shows the pertinent data from the nine cases in which toleration of nitrofurantoin was poor. There is no apparent correlation between drug intolerance and age, sex, race, or diagnosis.

Toxicity.—In eighteen cases serial nonprotein nitrogen determinations were done. The NPN rose in one case from 59 mg. to 95 mg. and in another from 147 mg. to 212 mg. per 100 cc., but in both instances the change probably was due to the underlying severe renal disease. Albumin appeared *de novo* in only 1 patient during therapy with nitrofurantoin, whereas in 15 cases with initial albuminemia there was either significantly less or no albumin after the treatment with nitrofurantoin. No casts or other evidences of renal irritation were found during or after therapy in any of the patients who did not have these elements in their urine prior to treatment. In no case was there evidence of depression of hemopoiesis due to nitrofurantoin. No skin rashes or other manifestations of hypersensitivity were encountered.

Four patients with severe systemic diseases died either during or shortly after therapy with nitrofurantoin. These patients had not responded to conventional therapy and were given the drug on the basis of a possible effect of nitrofurantoin on neoplastic cells.[16] The pertinent data from these cases

TABLE 7.—*Significant Data in Nine Patients in Whom Nitrofurantoin was Poorly Tolerated*

Age	Sex	Race	Diagnosis*	Daily Dosage, Mg.	Duration of Treatment, Days	Result
63	F	Negro	P	300	1 dose	Failure
62	M	White	C	300	6	Success
50	M	White	P	600	3	Undetermined
30	F	Negro	P	400	9	Success
68	M	White	P	300	58	Success
61	F	White	C	150	4	Undetermined
85	M	Negro	C	400	5	Undetermined
68	M	White	P	400	5	Failure
76	M	White	C	150	5	Undetermined

* C, cystitis; P, pyelonephritis.

TABLE 8.—*Results of Microscopic Examination of Organs of Four Patients Who Received Nitrofurantoin Prior to Death*

Age	Sex	Diagnosis	Duration of Therapy, Days	Route of Administration	Daily Dosage, Mg.	Total Dosage, Mg.	Heart	Kidney	Liver	Ovaries	Testicle
24	M	Tetratocarcinoma	18	Intravenous*	200	3600	N†	N	N	..	N‡
47	F	Metastatic carcinoma of the breast	36	Mouth	100	3600	N	N	N	N	..
67	M	Acute staphylococcic endocarditis	5	Intravenous	50	250	N	N	N	..	..
47	F	Systemic blastomycosis	90	Mouth	200	18	N	N	N	N	..

* For intravenous injection, the nitrofurantoin was dissolved in polyethylene glycol in a concentration of 6 mg. per milliliter.
† Normal.
‡ Testicular biopsy during therapy was also normal.

is shown in Table 8. No toxic effects on the testicles, kidney, liver, or ovaries were found (Table 8).

Clinical Results.—Therapy was successful in 27 cases; it failed in 12 cases and yielded inconclusive results in 21 cases. The criteria used to judge each case as successfully treated are presented in Table 9. In seven cases therapy was considered successful in spite of the persistence in the urine of bacteria that had been assumed to be causative organisms. However, we judged these cases as successfully treated because the object of therapy had been accomplished, i. e., to render the patient afebrile and to clear the urine. The relatively large number of inconclusive results was due to the type of patients treated and the strict criteria of objective evidence on the chart that we required in order to judge therapy a success. The number of times the various species of bacteria were eliminated from the urine, failed to respond to therapy, and appeared in the urine during or immediately after therapy are noted in Tables 10 and 11. The antimicrobial drugs which 14 of the 28 successfully treated patients had received prior to the administration of nitrofurantoin are listed in Table 12. Nitrofurantoin was the

TABLE 9.—*The Basis on Which It Was Decided That Treatment Was Successful in Twenty-Seven Cases* *

Case	Organism Not Present After Therapy	Temperature Response	Clearing of Urine	Clinical Improvement
1.....	0	0	+	+
2.....	+	+	+	+
3†.....	0	0	+	0
4.....	0	+	0	+
5.....	0	+	0	+
6.....	—	+	+	+
7.....	—	+	+	+
8.....	+	+	+	+
9.....	—	+	0	+
10.....	+	0	0	+
11.....	+	0	+	+
12.....	+	0	0	+
13.....	0	0	+	+
14.....	—	0	+	+
15.....	+	+	+	+
16.....	+	+	0	+
17.....	+	0	0	+
18.....	+	0	+	+
19.....	—	0	—	+‡
20.....	+	0	+	+
21.....	—	+	+	+
22.....	+	+	+	+
23.....	—	+	+	+
24.....	+	+	+	+
25.....	0	+	+	+
26.....	0	+	0	+
27.....	—	+	+	+

* 0, data not available or temperature normal before therapy; +, urine was cleared of pathogenic bacteria, temperature response occurred, urine was cleared by microscopic examination, or clinical improvement resulted; —, the condition at the top of the column did not occur.
† See Case 3 in the text.
‡ Marked dysuria and frequency disappeared.

TABLE 10.—*The Effectiveness of Nitrofurantoin Against Species of Pathogenic Bacteria Isolated Before Onset of Therapy*

Organism	Number of Times Therapy Eradicated the Organism	Number of Times Therapy Failed to Eradicate the Organism
Escherichia coli	15	5
Pseudomonas aeruginosa.	6	5
Pseudomonas species.....	2	1
Proteus	8	1
Micrococcus pyogenes.....	1	1
Paracolon bacillus.....	1	..
Total*.....	33	13

* The totals are greater than the number of patients because of mixed infections.

TABLE 11.—*Species of Bacteria That Appeared in Urine During or Immediately After Therapy with Nitrofurantoin That Were Not Present Before Therapy*

Species	Number of Times Each Species Was Found During or Immediately After Therapy
Pseudomonas aeruginosa	8
Nonhemolytic streptococci	4
Enterococci	2
Proteus	6
M. pyogenes	1

TABLE 12.—*Antimicrobial Agents Used Prior to Successful Therapy with Nitrofurantoin*

Drug	Number of Cases
Sulfisoxozole (Gantrisin)	5
Penicillin	4
Streptomycin	3
Oxytetracycline	2
Chlortetracycline	4
Erythromycin	1
Mandelic acid	1
Total	20*

* The 20 drugs were used in 14 cases.

initial therapy in the other 14 patients in whom treatment was a success.

REPORT OF CASES

CASE 1.—A 68-year-old man had had a surgical repair of a rectovesical fistula which had resulted from chronic diverticulitis. His postoperative course was complicated by wound dehiscence, infection, and evisceration. During the successful treatment of these complications he had received streptomycin, penicillin, chlortetracycline, chloramphenicol, erythromycin, and mandelic acid. After this therapy his wound healed well but he continued to have fever and pus and albumin in his urine. A urine culture revealed Proteus and P. aeruginosa. He was given, by mouth, 100 mg. of nitrofurantoin three times per day for six weeks. After two weeks of therapy with nitrofurantoin, his urine no longer contained either albumin or pus, repeated urine cultures grew only P. aeruginosa, and he was afebrile.

Comment.—The important feature of this case was the cure of a Proteus infection that had persisted in spite of therapy with antibiotics. In addition, a daily dose of 300 mg. of nitrofurantoin was given for six weeks with good toleration and no toxicity.

CASE 2.—A 76-year-old man entered the hospital because of bronchial pneumonia. Initial sputum cultures did not reveal a pathogenic organism. Penicillin and then oxytetracycline were administered, but the clinical response was equivocal. On the sixth hospital day, 50 mg. of nitrofurantoin was administered, by mouth, four times per day because a urine culture grew Proteus and P. aeruginosa. This was continued for six days. The patient then had a hard shaking chill and appeared acutely ill. A blood culture grew Proteus. This Proteus was found to be sensitive to 25γ/ml. of nitrofurantoin and to 6γ/ml. of chloramphenicol. The patient was given chloramphenicol and subsequently made an uneventful recovery.

Comment.—This case illustrates the truism that "there is no miracle drug." The proteus bacteremia developed during therapy with nitrofurantoin and responded to chloramphenicol which was the drug that in vitro studies indicated to be the one of choice. On the other hand, nitrofurantoin has been effective in some cases of bacteremia due to Proteus.[6] Thus laboratory control and more than one antimicrobial agent are necessary to cope with severe infections due to Proteus.

CASE 3.—A 41-year-old white man entered the hospital because of thrombophlebitis. The admission urinalysis revealed pyuria, and complete renal-function studies supported the diagnosis of chronic pyelonephritis that probably was superimposed on a chronic glomerular nephritis. Urine cultures grew only coagulase-negative staphylococci and gamma streptococci. The patient was started on long-term therapy with nitrofurantoin. The data regarding his subsequent urine studies are presented here in tabular form.

	1 Pretreatment Addis Count	2 Addis Count After Nitrofurantoin, 300 Mg/Day for 13 Days	3 Addis Count After Nitrofurantoin, 100 Mg/Day for 60 Days
RBC	15,000,000	8,000,000	9,000,000
WBC	363,000,000	112,000,000	26,000,000
Casts	1,000,000	378,000	350,000

The patient has remained asymptomatic and is continuing to take 100 mg. of nitrofurantoin daily, without evidence of toxicity or intolerance.

Comment.—This case is presented not as a proved cure (although it was classed as satisfactory) but as an example of the type of case in which nitrofurantoin should receive a thorough evaluation, i. e., chronic pyelonephritis in which long-term bactericidal therapy is indicated.

CASE 4.—A 59-year-old white woman entered the hospital because of gangrene of the foot that was associated with diabetes mellitus. She was febrile

and had frank pyuria, and a urine culture grew paracolon bacilli. Her condition remained unchanged in spite of therapy with oxytetracycline, chlortetracycline, and penicillin. One hundred milligrams of nitrofurantoin was administered by mouth, four times per day. Within 48 hours she was afebrile and her urine was free of pus and bacteria. Amputation then was performed, and the patient made an uneventful recovery.

Comment.—This is an example of a case in which nitrofurantoin effected a clinical and bacteriological cure whereas three antibiotics had been ineffective.

GENERAL COMMENT

Our clinical results and the findings of other workings are consistent with the lack of demonstrable nitrofurantoin-resistant mutants in our in vitro experiments.† The finding that large numbers of E. coli and Proteus organisms were resistant to nitrofurantoin whereas small numbers of the same strains were relatively sensitive suggests that these strains may produce a substance that inhibits nitrofurantoin.[12] In fact, a similar observation led to the demonstration that certain strains of E. coli produced a substance that inhibited polymyxin B.‡ Thus, a mode of bacterial resistance to nitrofurantoin may be the production of an inhibiting substance similar to penicillinase.

The finding that nitrofurantoin is relatively bactericidal is of particular importance because neomycin and streptomycin are the only others of the so-called "wide spectrum" antimicrobial agents that have this characteristic. Neomycin is too toxic for general use, and the rapid appearance of resistant mutants is a serious disadvantage of streptomycin, so nitrofurantoin may be unique as a wide-spectrum antimicrobial agent that is bactericidal, relatively nontoxic, and does not invoke resistant mutants.[18] The importance of an agent with these characteristics that could be used for a long period in the treatment of chronic pyelonephritis has been recognized, and it is in this sphere that nitrofurantoin may have its greatest use.[19] Another reason that nitrofurantoin merits the consideration of the clinician is that the in vitro studies that compared its activity with that of the antibiotics revealed that it was particularly effective against M. pyogenes and Proteus, which are among the species that are the most difficult to eradicate from the urinary tract.[20] These studies also showed that the spectrum of nitrofurantoin was most similar to that of chloramphenicol. However, in four cases nitrofurantoin was successful after chloramphenicol had failed and in several cases the reverse was true (Case 2). Therefore this similarity in spectrum should not discourage the clinician from changing from one to the other of these drugs if therapy is not succeeding.

Judged as a whole, the clinical results obtained with nitrofurantoin were satisfactory and in our opinion about equal to those we would have obtained using any other single drug. The number of unsuccessful and indeterminate results reflect the multiple difficulties of treating chronic urinary tract infections and not necessarily a lack of efficacy of nitrofurantoin. Fourteen of the 28 patients in whom treatment was successful had received other antimicrobial agents before treatment with nitrofurantoin. This suggests that nitrofurantoin may be tried with a fair chance of success in urinary tract infections that are not responding to therapy with other antimicrobial drugs.

Microscopic examination of the organs of patients who had received large dosages of nitrofurantoin was encouraging, since it revealed no evidence of toxic effects. In fact, we were unable to find any toxic effects that could have been attributed to nitrofurantoin. In spite of this, it should be remembered that animal experiments have shown that the nitrofurans inhibit spermatogenesis in young rats.[16] This possibility of testicular damage still must be borne in mind when young patients are treated. However, examination of the testicular tissue of our 24-year-old patient during and after therapy with nitrofurantoin and careful studies of biopsy material by other workers tend to indicate that this danger of testicular damage is not great.[2]

† References 2 and 17.

‡ References 11 and 12.

A particularly encouraging finding in this study was the fact that nitrofurantoin did not cause diarrhea in any of the patients. Since diarrhea is one of the most dangerous and troublesome aftermaths of antibiotic therapy, this might be a consideration in the choice of an antimicrobial drug, particularly if the patient is in the hospital.[20]

SUMMARY AND CONCLUSIONS

In vitro studies of the antibacterial activity of nitrofurantoin revealed that it has a wide antibacterial spectrum, is relatively bactericidal, and does not readily invoke resistant mutants. Nitrofurantoin was found particularly effective against M. pyogenes and Proteus and ineffective against P. aeruginosa.

Sixty patients with infections of the urinary tract were treated with nitrofurantoin. The results were satisfactory in 27 cases, unsatisfactory in 12 cases, and indeterminate in 21 cases. Fourteen of the 27 cases in whom treatment was effective had received prior unsuccessful therapy with antibiotics. In our opinion, these results were equal to or superior to those that would have been obtained with any other single antimicrobial agent. Nine patients had varying degrees of nausea and vomiting due to the nitrofurantoin, but no diarrhea or any evidence of toxicity was found.

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Use of Hyaluronidase by Iontophoresis in Treatment of Lymphedema

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Lymphedema is a swelling of soft tissues, the result of accumulation of increased quantities of lymph. A useful clinical classification by Allen, Barker, and Hines,[1] modified by Duryee,* divides all cases into two main groups; inflammatory and noninflammatory.

I. Noninflammatory
  A. Primary
    1. Congenital lymphedema
      (*a*) Hereditary or familial (Milroy's disease)
      (*b*) Lymphedema praecox
  B. Secondary
    1. Malignant occlusion
    2. Surgical removal of lymph nodes
    3. Pressure
    4. Radiation therapy
    5. Venous insufficiency

II. Inflammatory
  A. Primary (single or recurrent acute and chronic)
  B. Secondary (single or recurrent acute and chronic)
    1. Trichophytosis
    2. Systemic disease
    3. Local tissue injury or inflammation
    4. Filariasis

Lymphedema has been poorly responsive to numerous forms of treatment short of extensive surgery. For this reason, any method which might promise some improvement of the frequently disabling and disfiguring features of this condition, and which is clinically feasible, should be investigated.

Hyaluronidase, the "spreading factor," was first described in 1929 by Duran-Reynals.[2] Additional knowledge of its biological significance was contributed by Meyer.[3] The enzyme, found in sperm, bacteria, and tissue cells, especially those of the testis, hydrolyzes hyaluronic acid, the mucopolysaccharide gel which is present in interstitial tissue and which acts as a physical barrier to invasion by foreign material. Since the viscosity of the intercellular cement is reduced by hyaluronidase, fluids are more easily diffused and are more quickly absorbed into the general circulation. This action of hyaluronidase has been widely used to accelerate the diffusion and absorption of subcutaneously injected fluids and drugs.

Asboe-Hansen † used punch biopsies to demonstrate that the highly increased intercellular substance of myxedematous connective tissue is chiefly hyaluronic acid and that injection of hyaluronidase into such tissue reduces the extensive metachromatic intercellular substance. This finding has been confirmed by Rosman [6]; Bloom, Herrmann, and Sharlit [7]; Grais,[8] and Watson and Pearce.[9] Hyaluronidase injections have been used by Benzer and Schaffer [10] in the treatment of traumatic swelling of the face, head, and neck regions, and acceleration in reduc-

The hyaluronidase used in this study was provided as Wydase by Wyeth Laboratories, Inc., Philadelphia.

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* Duryee, A. W.: Personal communication to the author.

† References 4 and 5.

tion of the swellings and clearing of symptoms was reportedly dramatic.

It was reasoned that these characteristics of hyaluronidase might be employed in the disfiguring and frequently disabling lymphedema of upper or lower extremities, but the extensive nature of the swelling and the lowered resistance to infection of such lymphedematous tissue led to considerable reluctance to use hyaluronidase by injection. It was decided that administration by iontophoresis might solve both these objections, since it would thus be possible to treat large areas of body surface without pain or discomfort and the skin would be intact throughout. Hyaluronidase has been administered by iontophoresis by Popkin ‡ in the treatment of scleroderma and of chronic ulcers accompanying scleroderma, with evidence of efficacy.

There is at present no direct way to ascertain whether hyaluronidase administered by iontophoresis is actually absorbed through the skin. However, we were able to demonstrate that saline intradermal wheals are dissipated much more rapidly and completely from an area treated with hyaluronidase by iontophoresis than are identical wheals in the symmetrically opposed area treated by iontophoresis without hyaluronidase. In addition, we found that intradermal injections of 0.1 cc. of 0.4% Evans blue dye were dispersed by iontophoresis with hyaluronidase over an area at least four times greater in diameter than on the contralateral side similarly treated but without hyaluronidase as a control. This suggests that hyaluronidase administered by iontophoresis exerts an effect similar to that obtained by injection of hyaluronidase.

Using electrophoretic techniques, Hahn [18] established that at a pH of 5.2 hyaluronidase carries a positive electrical charge and migrates from the anode to the cathode. Tint § determined that the rate of migration was more rapid at a pH of 5.4.

‡ References 11 and 12.

§ Tint, H.: Personal communication to the author.

METHOD

The hyaluronidase was dissolved in 0.1 M acetate buffer solution, pH 5.4, which had the following formula:

Sodium acetate. $3H_2O$, 11.42 gm.
Glacial acetate acid, 0.923 cc.
Distilled water, q. s. 1000 cc.

At the start of this study, 1500 TRU (turbidity-reducing units) of hyaluronidase were added to 1 liter of acetate buffer solution. Later, 150 TRU were added to 250 cc. of buffer solution, and the

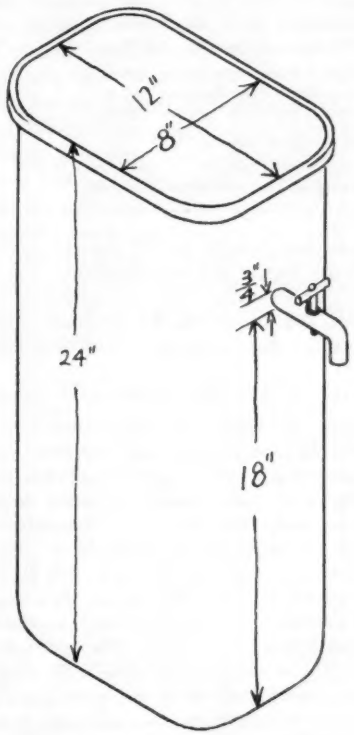

Fig. 1.—Tank used for volumetric measurements of extremities.

effectiveness seemed not to be diminished by the reduced concentration of hyaluronidase. Solutions were freshly prepared for the treatments, for the stability of hyaluronidase in solution is questionable. The acetate buffer may be prepared in stock solution.

The entire extremity to be treated was wrapped with diaper cloth soaked in the solution of hyaluronidase (or inert buffer solution, for control). A flexible block tin electrode was spiral-wrapped around the cloth, and the entire preparation was wrapped with a wet cotton-elastic bandage. This

TABLE 1.—*Case 1*

Date	Control or Hyaluronidase	Pre-treatment Volume, Cc.	Post-Treatment Volume, Cc.	Change, Cc.
5/22/52	C	5010	5000	— 10
5/24/52	C	4980	5010	+ 30
5/26/52	C	4960	4900	— 60
5/27/52	H	5030	4740	—290
5/30/52	H	4980	4700	—280
5/31/52	H	4720	4610	—110
6/ 2/52	H	4630	4480	—150
6/ 4/52	H	4740	4510	—230
6/ 6/52	H	4620	4530	— 90
6/ 8/52	H	4580	4340	—240
6/10/52	H	4470	4250	—220
6/12/52	H	4510	4280	—230
6/14/52	H	4370	4240	—130

Original volume, right leg	5010 cc.
Final volume, right leg	4240 cc.
Over-all reduction in volume	770 cc.
Mean change in volume	
3 control treatments	— 13.3 cc.
10 hyaluronidase treatments	—197.0 cc.

electrode was coupled to the positive pole of a 45-volt dry battery galvanic apparatus. The negative pole was coupled to a wet asbestos pad back electrode, and the current was gradually increased to 20 ma. and there maintained for 20 to 30 minutes.

METHOD OF DETERMINING EFFECT OF THERAPY

Because circumferential measurements of extremities by tape measure are extremely variable and inaccurate, a simple and reproducible method for volumetric measurements of either upper or lower extremity was devised. A flat-bottom tank (Fig. 1) of welded sheet metal, 24 in. (60 cm.) high, 8 in. (20 cm.) wide, and 12 in. (30 cm.) long, with rounded ends, was constructed. At a height of 18 in. (45 cm.), a ¾ in. (1.9 cm.) overflow tap, with faucet valve, was placed. The tank was filled to the level of the overflow valve with water at a temperature of 86 to 90 F; the valve was opened, and the extremity to be measured was immersed while the displaced water was caught and its volume measured. In measurements of the lower extremity the subject stood with foot flat on the bottom of the tank; for the upper extremity the tank was raised 18 in. (45 cm.) on a level platform, the arm was immersed with fist clenched, and the flat surface of the clenched fist was placed flat on the bottom of the tank. The variation between repeat readings did not exceed 10 cc.; therefore the device was considered suitably accurate.

Volumetric measurements were done on the lower extremities of two normal subjects without known circulatory pathology to determine the extent of fluctuation in volume on separate occasions. Variations of as much as 200 cc. in extremity volume were found on different days, seemingly related to environmental temperature. However, repeated measurements on the same day showed variations of no more than ±10 cc. Measurements on the same normal subjects before and after elevation of the lower extremity for 30 minutes, with and without accompanying acetate buffer iontophoresis, on repeated occasions, showed a maximum diminution in volume of 30 cc.

REPORT OF CASES

CASE 1 (Table 1).—P. H., a 66-year-old white male tailor, who had numerous attacks of recurrent acute superficial and deep thrombophlebitis of the right leg, starting at about 40 years of age, was the first patient on whom this treatment was attempted. He was admitted to the University Hospital for this purpose.

For more than 18 years he had been handicapped by chronic lymphedema of the right leg, which had been treated for more than 8 years with constrictive support in the form of wide cotton elastic bandage, elastic stocking, or laced canvas boot, without any

TABLE 2.—*Case 2*

Date	Control or Hyaluronidase	Pre-treatment Volume, Cc.	Post-Treatment Volume, Cc.	Change, Cc.
12/29/52	H	5320	5180	—140
1/ 5/53	H	5320	5010	—310
1/12/53	C	4810	4800	— 10
1/19/53	H	4950	5000	+ 50
1/26/53	H	4850	4760	— 90
2/ 2/53	H	4910	4910	0
2/ 9/53	H	4890	4710	—180
2/16/53	H	4720	4550	—170
3/ 2/53	H	4730	4580	—150
3/ 9/53	H	4590	4450	—140
3/16/53	H	4590	4500	— 90
3/23/53	H	4560	4360	—200
3/30/53	C	4480	4460	— 20
4/ 6/53	C	4520	4500	— 20
4/12/53	C	4490	4460	— 30
4/20/53	H	4450	4260	—190
4/27/53	H	4360	4200	—160
5/ 4/53	H	4450	4310	—140
5/11/53	H	4520	4440	— 80
5/18/53	H	4420	4310	—110
5/25/53	H	4600	4480	—120
6/ 1/53	H	4490	4290	—200
6/ 8/53	H	4540	4470	— 70
6/15/53	H	4520	4410	—110
6/22/53	H	4490	4320	—170
6/29/53	H	4430	4280	—150
7/ 6/53	H	4390	4210	—180
7/13/53	H	4420	4240	—180
7/20/53	H	4380	4180	—200
7/27/53	H	4350	4170	—180

Original volume, right leg	5320 cc.
Final volume, right leg	4170 cc.
Over-all reduction in volume	1150 cc.
Mean change in volume	
4 control treatments	— 20 cc.
26 hyaluronidase treatments	—140 cc.

TABLE 3.—*Case 3*

Date	Control or Hyaluronidase	Pre-treatment Volume, Cc.	Post-Treatment Volume, Cc.	Change, Cc.
10/17/52	H	4790	4380	—410
11/ 3/52	H	4830	4320	—510
11/10/52	H	4720	4480	—240
11/17/52	H	4930	4710	—220
11/24/52	C	4910	4860	— 50
12/ 1/52	C	4650	4630	— 20
12/ 8/52	H	4950	4720	—230
12/15/52	H	4990	4670	—320
12/22/52	H	4800	4680	—120
12/29/52	H	4890	4740	—150
1/ 5/53	H	4810	4780	— 30
1/12/53	H	4780	4550	—230
1/19/53	C	4680	4650	— 30
1/26/53	C	4610	4610	0
2/ 2/53	H	4820	4700	—120
2/ 9/53	C	4810	4820	+ 10
2/16/53	H	4720	4560	—160
2/23/53	H	4810	4660	—150
3/ 2/53	H	4910	4740	—170
3/ 9/53	H	4990	4600	—390
3/23/53	H	4890	4540	—350
3/30/53	C	4910	4900	— 10
4/ 6/53	C	4800	4780	— 20
4/13/53	H	4840	4620	—220
4/20/53	H	4930	4640	—290
4/27/53	H	4940	4700	—240
5/ 4/53	H	4870	4640	—230
5/11/53	H	4920	4690	—230
5/18/53	H	4750	4600	—150
5/25/53	H	4920	4890	— 30
6/ 1/53	H	4850	4700	—150
6/ 8/53	H	4940	4790	—150
6/15/53	H	5030	4930	—100
6/22/53	H	5000	4700	—300
6/29/53	H	4990	4680	—310
7/ 6/53	H	4900	4690	—210
7/13/53	C	4990	4980	— 10
7/20/53	H	5130	4800	—330
7/27/53	H	5050	4700	—350

Original volume, left arm	4790 cc.
Final volume, left arm	4700 cc.
Over-all reduction in volume	90 cc.
Mean change in volume	
8 control treatments	— 16.3 cc.
31 hyaluronidase treatments	—228.7 cc.

evident improvement. He had also been through three periods of four to six weeks of bed rest with elevation of the lower extremities, without apparent diminution in size of the affected extremity.

This patient was begun on control iontophoresis treatments without hyaluronidase, and no significant alterations in volume occurred after three such treatments. He was then started on iontophoresis with hyaluronidase, and perceptible and progressive reduction in size of his right leg resulted.

After 10 active treatments, he was able to return to work, and he has remained actively employed since, with the use of a 4 in. (10 cm.) wide elastic bandage being sufficient to prevent reaccumulation of fluid.

CASE 2 (Table 2).—E. G., a 48-year-old white male accountant, had acute thrombophlebitis of the right calf three years ago. He was hospitalized at New York Hospital and treated with penicillin for 10 days. Swelling of the right leg appeared and increased in degree in spite of faithful use of elastic bandage for support. Six months later an indolent ulcer developed on the medial aspect of the right ankle. He was treated with an ointment containing 2% pantothenyl alcohol (Panthoderm ointment) and elastic bandage compression support. An eczematoid eruption appeared over his entire right leg up to 6 in. (15 cm.) above the knee. The lymphedema persisted, although the ulcer slowly healed over a period of nearly two years, but the eczematoid eruption remained without improvement until the present course of treatment.

This patient received 24 treatments with hyaluronidase by iontophoresis at weekly intervals, with 4 control treatments interspersed. There was a striking diminution in volume of the leg, and by April 27, 1953, after 13 active treatments, the eczematoid eruption had completely healed, and the skin remained intact thereafter. An elastic bandage was faithfully worn during the intervals between treatments. This man was also able to resume gainful employment.

TABLE 4.—*Case 4*

Date	Control or Hyaluronidase	Pre-treatment Volume, Cc.	Post-Treatment Volume, Cc.	Change, Cc.
9/ 1/53	C	7140	7120	— 20
9/ 8/53	C	7210	7200	— 10
9/15/53	H	7180	7010	—170
9/22/53	H	7030	6890	—140
9/29/53	H	7010	6720	—290
10/ 6/53	H	6980	6610	—380
10/13/53	C	6820	6790	— 30
10/20/53	H	6720	6420	—300
10/27/53	H	6570	6290	—280
11/ 3/53	H	6640	6220	—420
11/10/53	H	6530	6140	—390
11/17/53	H	6280	5970	—310
11/23/53	H	6040	5720	—320
12/ 1/53	H	5950	5480	—470
12/ 8/53	H	5830	5510	—320
12/15/53	H	5580	5320	—260
12/22/53	H	5280	5190	— 90
12/29/53	C	5340	5310	— 30
1/ 5/54	H	5470	5120	—350
1/12/54	H	5330	5070	—260
1/19/54	H	5420	5240	—180
1/26/54	H	5370	5080	—290

Original volume, left leg	7140 cc.
Final volume, left leg	5080 cc.
Over-all reduction in volume	2060 cc.
Mean change in volume	
4 control treatments	— 22.5 cc.
18 hyaluronidase treatments	—290.0 cc.

TABLE 5.—*Case 5*

Date	Control or Hyaluronidase	Pre-treatment Volume, Cc.	Post-Treatment Volume, Cc.	Change, Cc.
9/ 1/53	C	5890	5890	0
9/ 8/53	C	5920	5890	— 30
9/15/53	H	5940	5780	—160
9/22/53	H	5850	5620	—230
9/29/53	H	5780	5540	—240
10/ 6/53	H	5720	5460	—260
10/13/53	C	5840	5800	— 40
10/20/53	H	5790	5510	—280
10/27/53	H	5680	5420	—260
11/ 3/53	H	5470	5160	—310
11/10/53	H	5240	5010	—230
11/17/53	H	5270	4950	—320
11/24/53	H	5090	4740	—350
12/ 1/53	H	5120	4790	—330
12/ 8/53	H	5020	4680	—340
12/15/53	H	4860	4590	—270
12/22/53	H	4820	4560	—260
12/29/53	C	4930	4920	— 10
1/ 5/54	H	4940	4730	—210
1/12/54	H	4710	4530	—180
1/19/54	H	4760	4510	—250
1/26/54	H	4820	4520	—300

Original volume, right leg.. 5890 cc.
Final volume, right leg..... 4520 cc.
Over-all reduction in volume 2060 cc.

Mean change in volume

4 control treatments..... — 20 cc.
18 hyaluronidase treatments —265 cc.

CASE 3 (Table 3).—F. J., a 72-year-old white housewife, had a left radical mastectomy with axillary dissection for breast carcinoma at University Hospital in July, 1948. Following surgery, there developed gradually increasing massive lymphedema of the left arm, which did not recede. She received 31 treatments with hyaluronidase plus 8 control treatments, but she could not be persuaded to use elastic bandage or binder on the arm between treatments. She had marked reduction in volume of the arm as a result of most of the active treatments, but no progressive reduction could be achieved, possibly because of her refusal to wear elastic support. This patient experienced a copious diuresis for many hours following each active treatment.

CASE 4 (Table 4).—S. G., a 54-year-old white dressmaker, had massive lymphedema praecox of the left leg, with onset at the age of 12, at about the same time as onset of the menarche. She exhibited another vascular anomaly in the form of a congenital lymphangioma covering the entire left side of her face.

Many forms of treatment had been tried to reduce the swelling of her left leg, which was truly massive, but there had never been even slight or transient improvement. She was given 4 control iontophoresis treatments and 18 treatments with hyaluronidase, with startling and dramatic reduction in size of the left lower extremity. She cooperated enthusiastically, wearing firm cotton elastic bandage support on her left leg during all waking moments between treatments.

For several weeks, the problem of laxness of the skin after loss of fluid was prominent, but with continuation of firm constrictive bandaging, shrinkage of the skin and normalization of the contours of the extremity became apparent. This patient also experienced copious diuresis for many hours following each active treatment.

Although marked reduction in size of the left leg was effected, it still was markedly larger than the normal right leg at the conclusion of treatment. The difference in volume between the two extremities was 1400 cc.

CASE 5 (Table 5).—J. W., a 56-year-old white housewife, had a severe cellulitis and lymphangitis of the right foot and leg at the age of 15. Swelling

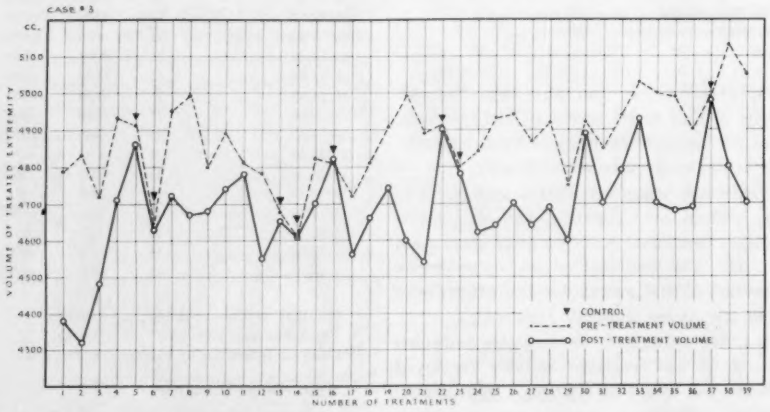

Fig. 2 (Case 3).—Response to treatments of left upper extremity with hyaluronidase by iontophoresis.

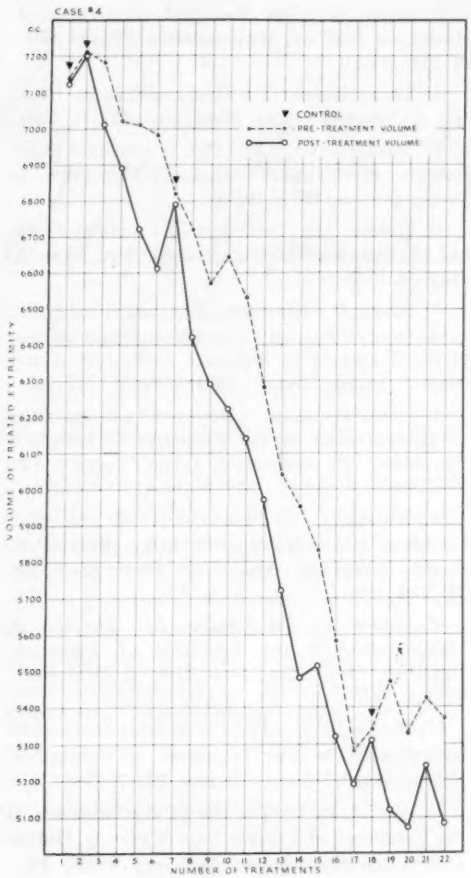

Fig. 3 (Case 4).—Response to treatments of left lower extremity with hyaluronidase by iontophoresis.

of the right leg appeared and increased gradually over a period of many years, in spite of diligent use of elastic supports and varied forms of treatment.

She received 4 control treatments and 18 treatments with hyaluronidase by iontophoresis, with definite and progressive diminution in volume of her right leg, so that at the conclusion of these treatments her right leg was volumetrically only 480 cc. larger than her left.

COMMENT

A favorable response to treatment with hyaluronidase by iontophoresis was achieved in five patients with lymphedema of upper or lower extremities of varied etiology. In four of these five patients, the reduction in volume of the treated extremity was progressive and was maintained by simple elastic bandage compression. In the remaining patient, Case 3 (Table 3; Fig. 2), the upper extremity was involved, and the patient could not be induced to wear constrictive support for her arm during the intervals between treatments. It is noteworthy that the diminution in volume of her extremity during the active treatments was comparable with that achieved in the other patients, but the diminution in volume could not be maintained. It seems probable that this was due to failure to use constrictive elastic support during the intervals between treatments. A useful elastic sleeve for the upper extremity has been described by Foley [14] and might be used to good advantage in conjunction with hyaluronidase by iontophoresis. The most dramatic and progressive diminution in volume of an involved extremity was noted in Case 4 (Table 4; Fig. 3).

In Table 6 is shown the marked difference in response to control treatments and to treatments with hyaluronidase.

The occurrence of profuse diuresis in two of the patients (Cases 3 and 4) deserves mention. No other side-effects or untoward reactions to the treatments were noted. The only subjective sensations during the treatments consisted of tingling of the treated extremity or of the back. After the treatments there was marked alleviation of the sensation of heaviness of the involved extremity in all patients.

SUMMARY AND CONCLUSIONS

A method of administration of hyaluronidase by iontophoresis is described.

TABLE 6.—*Comparative Response to Treatments*

	Control Treatments		Hyaluronidase Treatments	
Case	No.	Mean Change in Volume, Cc.	No.	Mean Change in Volume, Cc.
1.....	4	—13.3	10	—197.0
2.....	4	—20.0	26	—140.0
3.....	8	—16.3	81	—228.7
4.....	4	—22.3	18	—290.0
5.....	4	—20.0	18	—265.0
Means.....	4.8	—18.4	20.6	—224.1

This method has been used to determine its effect on lymphedema of upper and lower extremities.

It has been demonstrated that hyaluronidase causes increased absorption of fluid from the skin and subcutaneous tissues when administered by iontophoresis.

After a treatment it can be shown by volumetric measurements that there is significant movement of lymphatic fluid, with diminution of limb volume, during a single 30-minute treatment.

Use of constrictive elastic supports during the intervals between treatments appears to aid in accomplishing progressive diminution in volume of a lymphedematous extremity.

No untoward reactions were observed in this series of 103 treatments with hyaluronidase by iontophoresis.

Study of a larger group of patients suffering from lymphedema, with therapy over an extended period, will be necessary to determine whether prolonged and persistent reduction of lymphedema can be accomplished by this method.

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Apparent Hepatic Dysfunction in Lupus Erythematosus

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Abnormality of hepatic function has been described in a number of disorders that do not involve the liver primarily.* In rheumatoid arthritis,† subacute bacterial endocarditis,‡ sarcoidosis,§ convalescent malaria,‖ and other diseases characterized by increased serum levels of gamma globulin, this is manifested most prominently in the flocculation tests of liver function. Other tests considered to measure parenchymal hepatic function may show little deviation from the normal in patients with these diseases.[8]

There have been scattered references to the occurrence of abnormality of the thymol turbidity and cephalin flocculation in patients with disseminated lupus erythematosus.‖ A number of observers ¶ have reported that the gamma globulin fraction of the serum of patients with this disease is elevated. The present study of patients with lupus erythematosus is one of a series of studies of hepatic function in nonhepatic disease. Observations on other interesting and possibly related serological phenomena in lupus erythematosus are included.

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* References 1-7.

† References 2, 6, and 7.

‡ References 2, 6, and 8.

§ References 9 and 10.

‖ References 2, 6, 7, and 12-15.

¶ References 15-19.

METHODS AND PROCEDURE

Twenty-five patients were studied (Table 1). There were, in all of the patients, characteristic clinical features and skin lesions or biopsy findings, consistent with lupus erythematosus. In 21 of the 25, the clinical diagnosis was supported by demonstrating the L. E. phenomenon on one or more occasions. Six patients were studied at autopsy. Five of these showed the histologic changes characteristic of lupus erythematosus. In one, histologic examination revealed periarteritis nodosa. Nevertheless, this patient is included, since the clinical features were consistent with the observations in the remainder of the group and the L. E. test was positive. The histologic characteristics of the liver were evaluated in the six patients who were studied at autopsy and in five patients from whom specimens of the liver were obtained by punch biopsy.

The following tests of hepatic function were performed; sulfobromophthalein (Bromsulphalein) excretion by the method of Mateer and his associates,[20] the thymol turbidity by the procedure of Maclagan [21] as modified by Shank and Hoagland,[22] and the cephalin-cholesterol flocculation by Hanger's [23] method, with the modification of Neefe and Rhinhold.[24] Total serum proteins and the albumin and globulin levels were determined by the method of Howe,[25] with the modification described by Hiller,[26] and the alkaline phosphatase levels were determined by the method of Bodansky.[27] Electrophoretic patterns were determined in two patients with the Tiselius procedure. The serum bilirubin level and partition was done by the method of Molloy and Evelyn.[28] The other laboratory data analyzed in this study included the blood leucocyte count, the Wassermann and Kahn serologic tests for syphilis (23 patients), colloidal gold test of the spinal fluid (2 patients), a search for cold agglutinins (7 patients), determination of cryoglobulins (5 patients), and the results obtained with the Coombs test (8 patients).

TABLE 1.—*Summary of Data on Twenty-Five Patients with Disseminated Lupus Erythematosus*

Patient	Age, Yr.	Race	Sex	Method of Diagnosis	Thymol Turbidity, Units	Cephalin Flocculation	Albumin, Gm./100 Cc.	Globulin, Gm./100 Cc.	Sulfobromophthalein Excretion, %	Alkaline Phosphatase, Units	Total Bilirubin, Mg.	Direct-Reacting Bilirubin, Mg.	Hepatomegaly Splenomegaly	Jaundice	Peripheral Leucocyte Count	STS	Direct Coombs Test	Indirect Coombs Test	Cold Agglutinins	Cryoglobulin	Hemoglobin, Gm.	Acquired Hemolytic Anemia
I. R. B.	32	W	F	Clinical	11	0	4.4	2.7	....	11.2	0.5	0.1	0 0	0	Normal	Neg.	....	....	....	....	14.5	0
J. F.	11	W	M	L. E. cells	12	0	2.8	3.2	....	....	7	4.1	+ 0	+	3600	Neg.	....	....	....	....	10.5	0
G. G.	16	W	F	L. E. cells	15	4+	2.6	3.5	0.6	....	0.3	....	++	0	3800	16 K. U.	....	....	....	....	7.5	0
F. G.	16	W	F	L. E. cells	5.5	3+	3.6	2.6	....	....	0.5	0	0 0	0	1800	Neg.	....	....	....	....	5.8	0
W. H.	10	W	M	L. E. cells	7	4+	2.3	4.0	11	4.0	....	....	+ 0	0	Normal	....	....	....	....	....	13.4	0
D. H.	7	W	F	L. E. cells	22.9	3+	3.9	4.6	1.4	2.1	....	....	0 0	0	4200	....	....	....	....	....	10.7	0
B. J.	11	N	F	L. E. cells	25	4+	2.9	4.4	....	....	0.37	0.2	0 0	0	Normal	Neg.	....	....	....	....	10.8	0
C. K.	30	W	M	L. E. cells	9.3	0	5.2	3.1	4.7	1.0	1.2	0.28	0 0	0	4300	Neg.	....	....	....	....	16.7	0
I. K.	31	W	F	L. E. cells	23	4+	3.4	4.3	0	2.9	1.6	....	0 +	0	2500	Neg.	+	+	1:128	....	3.4	+
M. K.	17	W	F	L. E. cells	11.8	4+	1.8	3.2	....	8.0	14.0	7.4	+ 0	+	4300	Neg.	Neg.	Neg.	Neg.	Neg.	9.0	0
W. M.	33	N	F	Clinical	18.5	0	3.1	3.9	13.5	....	0.5	0	+ 0	0	2400	Neg.	....	....	....	....	8.7	0
H. M.	25	N	F	L. E. cells	27.5	2+	3.1	6.9	....	....	1.2	....	+ 0	0	4000	256 K. U.	....	....	1:512	4+	7.5	0
E. M.	20	N	F	L. E. cells	35	0	2.9	6.1	8.3	4.9	0.1	....	0 0	0	4.9	Neg.	Neg.	+	Neg.	Neg.	8.5	0
R. N.	..	N	F	L. E. cells	....	...	2.7	8.1	....	....	....	....	++	0	Normal	64 K. U.	+	Neg.	....	....	7.5	+
W. N.	44	W	M	L. E. cells	....	...	4.4	2.4	2.5	4.1	0.3	....	+ 0	+	11.7	4 K. U.	....	....	....	....	6.0	0
C. P.	22	W	F	L. E. cells	24.5	4+	4.3	3.7	25	2.4	0.2	0.1	+ 0	0	Normal	Neg.	Neg.?	Neg.	1:32	2+	13.0	0
C. P.	9	N	F	Clinical	17	4+	2.9	6.0	3.1	....	0.64	0.18	0 0	0	4.0	Neg.	....	....	....	....	9.1	0
T. H.	64	W	M	L. E. cells	12.0	4+	1.2	6.0	6	0.6	0.6	....	++	..	Normal	Neg.	....	....	1:128	....	12.0	0
S. T.	49	N	F	Clinical	6.2	0	3.5	4.0	5	3.3	0.5	0.3	0 0	0	Normal	Neg.	....	....	....	....	9.2	0
J. V.	11	W	M	L. E. cells	18.5	4+	2.6	4.2	1.5	3.5	1.5	0.6	0 0	0	2.6	Neg.	+	+	....	....	5.6	+
M. W.	19	N	F	L. E. cells	22.3	4+	3.1	5.3	7.1	2.7	0.5	0.05	0 0	0	15.8	Neg.	Neg.	Neg.	Neg.	Neg.	10.2	0
R. W.	15	W	F	L. E. cells	28.0	0	3.1	3.1	....	5.0	0.3	0.05	0 0	0	1.7	Neg.	....	....	....	....	9.0	0
A. C.	57	W	M	L. E. cells	16.4	4+	2.2	3.8	8.5	1.5	0.5	....	0 +	0	4.3	Neg.	Neg.	Neg.	....	....	12.0	0
H. K.	28	W	F	L. E. cells	19.5	3+	4.4	3.6	1.4	....	0.34	....	0 0	0	15.2	Neg.	....	....	....	....	12.5	0
M. S.*	49	W	F	L. E. cells	20.5	4+	2.7	5.0	13.5	3.4	0.5	0.2	+ 0	0	3.2	Neg.	....	....	....	....	10.3	0

* This patient had a clinical diagnosis of lupus erythematosus, with a positive L. E. test, but an autopsy diagnosis of periarteritis nodosa

TABLE 2.—*Incidence of Abnormality of Liver-Function Tests*

Test	Patients, No.	Patients with Abnormal Tests, %	Tests, No.	Abnormal Tests, %	Mean Value	Most Abnormal Values	Abnormal Values
Thymol turbidity	23	100	51	92	17.6	35	>4 units
Cephalin flocculation.....	23	70	50	64	....	4+	>2+ in 48 hr.
Albumin	25	80	..	..	....	1.8	<4 gm.
Globulin	25	88	..	..	....	3.1	>3 gm.
Sulfobromophthalein	17	47	21	38	6.6	25.0	>5% in 45 min.
Alkaline phosphatase....	16	31	24	29	3.8	11.2	>4 Bodansky units
Total bilirubin.....	22	27	..	..	....	14.0	>1.0
Direct-reacting bilirubin.	18	16	..	..	....	7.1	>0.2
Hepatomegaly	25	52	..	..	....	....	.....
Jaundice	25	12	..	..	....	....	.....

RESULTS

Abnormal results with one or more tests of hepatic function were obtained in all of the patients studied (Tables 1 and 2). Of the tests used, the thymol turbidity showed the highest incidence of abnormality. This procedure was performed 51 times in 23 patients, yielding elevated values in 47 of the 51 determinations. In each patient, however, an abnormal level was found on at least one occasion. The mean value of the thymol turbidity was 17.6 units; the highest value, 35 units. Abnormality of the cephalin-flocculation test was somewhat less frequent (70%). Sulfobromophthalein excretion was abnormal in 47% of the group, but in only one patient (C. P.) was the degree of abnormality more than minimal. In one-third of the patients studied there was elevation of the alkaline phosphatase level, with the highest value being 11.2 Bodansky units. Elevation of the serum bilirubin level occurred in 27% of the patients, but in only two of these patients was the direct-reacting fraction significantly abnormal. Jaundice was noted in 3 and hepatomegaly in 10 of the 25 patients.

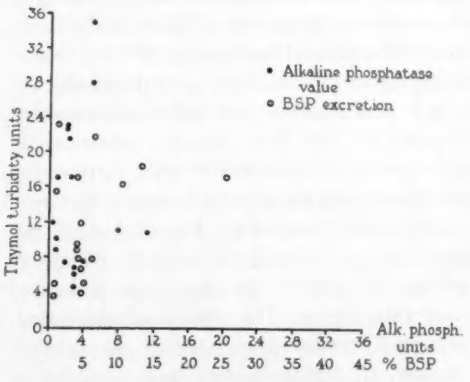


Fig. 1.—Comparison of thymol turbidity with alkaline phosphatase value and sulfobromophthalein excretion.

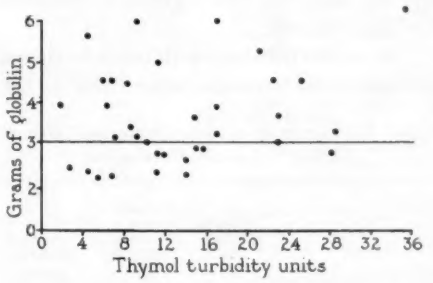


Fig. 2.—Relationship of thymol turbidity to total globulin.

In Figure 1 are plotted the values for the degree of sulfobromophthalein retention and for the alkaline phosphatase level, against the thymol turbidity values. As may be seen from this graph, marked abnormality of the thymol turbidity was associated with little or no abnormality of the dye excretion test or of the alkaline phosphatase level.

Elevation of the serum globulin was observed in 88% of the patients, whereas the serum albumin level was decreased in 80% of the group. Plotting the values for the serum globulin against the thymol turbidity levels (Fig. 2) revealed a poor correlation between the two. Values of the thymol turbidity above 16 units, however, were associated in all but one instance with elevation of the serum globulin level.

The values obtained by electrophoretic analysis of the serum of two patients are

TABLE 3.—*Results of Electrophoretic Analysis of Proteins in Two Patients with Lupus Erythematosus*

	Albumin, %*	Globulin			
		Alpha$_1$	Alpha$_2$	Beta	Gamma
Normal Values	53-59	6-8.5	6-11	11-15.6	12-17
I. K.					
2/ 6/51	46.4	3.6	6.1	15.6	28.3
3/20/51	50.5	3.8	5.5	8.2	31.9
4/23/51	37.4	6.8	8.6	11.3	35.9
E. M.					
7/21/53	26.0	4.6	9.4	11.4	48.6

* Values are expressed as per cent of total protein.

shown in Table 3. The gamma globulin was markedly elevated; the albumin level, depressed, and the alpha-1-globulin, somewhat less than normal.

The results obtained with other laboratory procedures are summarized in Table 4. Leucopenia (levels below 5000 per cc.) was noted, at sometime during the course of the disease, in only 60% of the patients. In some of these patients repeated determinations of the leucocyte level had to be performed before leucopenia could be demonstrated. A biologic false positive test for syphilis was demonstrated in 18% of the patients. In one patient, whose serum gave a value of 256 Kahn units, the spinal fluid showed a "first zone" colloidal gold curve and an elevated protein level on several examinations. This patient, who on postmortem examination showed no evidence of syphilis, was the one with the histologic diagnosis of periarteritis nodosa.

TABLE 4.—*Incidence of Leucopenia and Miscellaneous Serologic Phenomena*

Test	Patients Studied, No.	Patients with Abnormal Tests, %	Range of Values
Leucopenia	25	60	1.7-20.0
Serology	23	18	0-256 Kahn Units
Coombs test	8*	50	.....
Cold agglutinins	7	43	0-1:512
Cryoglobulin	5	40	0-4+
Acquired hemolytic anemia	25	12	.....

* Of the four positive results with the Coombs test, two were obtained by the "direct" and two by the "indirect" technique.

The direct Coombs test was performed in eight patients. Three of these showed clinical and laboratory evidence of hemolytic anemia, and in these patients the results of the test were positive. In one without evidence of hemolysis, the indirect Coombs test was positive but the direct test was negative. An elevated level of cold agglutinins was found in three of seven patients studied. Cryoglobulins were sought in the serum of five patients, and, in two of these, were demonstrated. One of these patients had gangrene of the fingers.

Microscopic studies of the liver were available in five biopsy specimens and in six autopsy specimens. No consistent histologic abnormalities were noted. Several nonspecific deviations from the normal were seen in the autopsy specimens (Table 5). These included scattered small areas of focal necrosis, lymphocytic infiltration in the portal regions, proliferation and mild inflammatory changes of the liver capsule, questionable prominence of the Kupffer cells, fatty metamorphosis, congestion, and, in one instance, centrolobular necrosis. In all but 2 patients of the 11 studied, the fatty metamorphosis was slight. In these two, it was of moderate degree. The sinusoidal congestion, seen in all six autopsied patients, was marked in one, moderate in two, and minimal in three. Small scattered granulomas were seen in the liver tissue of one patient (Fig. 3).

Fig. 3 (Patient M. K., Table 5).—Photomicrograph showing granuloma of liver.

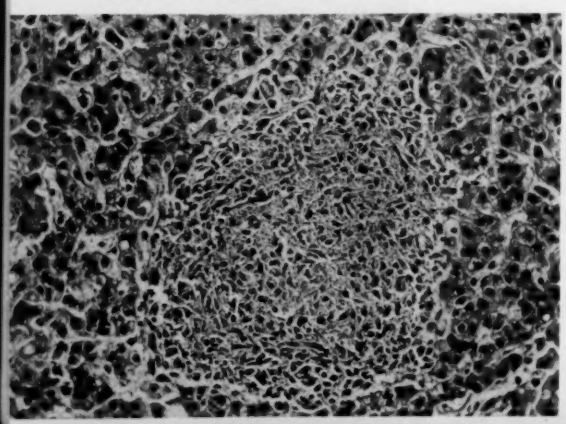


TABLE 5.—*Correlation of Studies of Hepatic Function with Histologic Data*

Patient	Thymol Turbidity, Units	Cephalin Flocculation	Albumin, Gm.	Globulin, Gm.	Sulfobromophthalein, %	Alkaline Phosphatase, Units	Total Bilirubin	Direct-Reacting Bilirubin	Histologic Changes
W. H. Autopsy	7	4+	2.3	4.0	11	4.0	...	...	Mild congestion; marked thickening of a few portal arterioles and small arteries
M. K. Autopsy	11.8	4+	1.8	3.2	...	8.0	14.0	7.4	Mild focal fatty changes; marked bile stasis; small focal intralobular granulomas; mild congestion
E. M. Autopsy	35	0	2.9	6.1	8.3	4.9	0.1	...	Moderate congestion with fatty changes largely in central region; subcapsular inflammation
H. M.* Autopsy	27.5	2+	3.1	6.9	...	...	1.2	...	Moderate focal fatty changes; mild congestion; healing and healed periarteritis lesions with areas of infarction
C. P. Autopsy	24.5	4+	4.3	3.7	25	2.4	0.2	0.1	Marked chronic passive congestion with central fatty changes
J. V. Autopsy	18.5	4+	2.6	4.2	1.5	3.5	1.5	0.6	Mild congestion; mild focal fatty changes; scattered small focal necrosis
C. K. Biopsy	9.3	0	5.2	3.1	4.7	1.0	1.2	0.28	Moderate fatty metamorphosis; mild capsular fibrosis; portal infiltration
H. K. Biopsy	12.0	3+	4.4	3.0	1.4	...	0.2	...	Portal infiltration
M. S. Biopsy	7.1	4+	3.2	2.7	4.5	...	0.5	0.2	Mild fatty changes; portal infiltration
T. H. Biopsy	12.0	4+	3.2	5.0	5	0.6	...	...	Mild portal inflammation
A. C. Biopsy	9.3	4+	2.2	3.4	8.5	1.5	0.5	...	Mild portal infiltration

* In this patient the autopsy diagnosis was periarteritis nodosa.

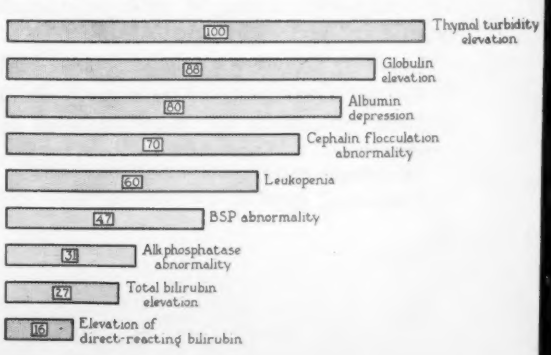
It is of interest to note that this patient (M. K.) had clinical jaundice and evidence of bile stasis in the histologic sections. Neither in this patient nor in the others was there evidence of hepatic necrosis. In the five biopsy tissues studied, only portal inflammatory infiltration was observed. In all instances the biopsy was performed within two weeks of the day on which the studies of hepatic function were obtained.

COMMENT

In all patients studied, abnormality of one or more tests of hepatic function was noted (Fig. 4). This is in general agreement with previous reports # that elevation of the thymol turbidity occurs in patients with lupus erythematosus. It seems unlikely, however, that these results measure hepatic dysfunction. Extravagant degrees of abnormality of the thymol turbidity value, with levels as high as 20 to 35 units, were associated with normal or meagerly abnormal levels of sulfobromophthalein retention. Furthermore, histologic studies of the liver in this group of patients with lupus erythematosus failed to reveal consistent changes in the hepatic parenchyma. Studies by Klemperer and co-

References 2, 6, 7, and 12-15.

Fig. 4.—Relative incidence of abnormality of various tests of hepatic function compared with incidence of leucopenia in 25 patients with disseminated lupus erythematosus.



workers [29] and by Mortenson and Gormsen [12] have likewise failed to reveal significant parenchymatous changes in the liver. The observation of a small granuloma in 1 patient of the 12 whose livers were studied may reasonably be considered a part of the systemic disease. Teilum [30] discussed "miliary epitheloid granulomas" of the serous membranes, lungs, and lymph nodes of two patients with disseminated lupus erythematosus. It therefore seems reasonable to assume that the abnormality of the flocculation tests in these patients probably was related to the disturbance in plasma proteins and was not primarily evidence of impaired hepatic function. The high incidence of hyperglobulinemia and of hypoalbuminemia is in agreement with the observations of others.* That the depressed serum albumin levels may represent hepatic dysfunction cannot be excluded. Nevertheless, this finding is sufficiently nonspecific and is associated with so many chronic and debilitating diseases that it cannot be considered a precise or direct index of abnormality of the hepatic parenchyma.

Actual hepatic damage has to be assumed in Patients J. F. and M. K., who had jaundice of the hepatocellular type and significantly abnormal sulfobromophthalein excretion, alkaline phosphatase levels, and abnormal flocculation tests. This may also be true of Patients W. H. and C. P., whose sulfobromophthalein excretion was significantly abnormal. The histologic demonstration of marked chronic passive congestion of the liver in Patient C. P. may, however, be related to the inability to clear the dye, since abnormality of this hepatic function has been demonstrated in congestive heart failure.[31]

The discrepancy between the incidence of abnormality of the thymol-turbidity and the cephalin-flocculation test is of interest. It has been shown in many other diseases, both hepatic and nonhepatic, that abnormality of one of these procedures may be present with or without abnormality of the other.† Both of these procedures are more likely to be abnormal in the presence of elevated levels of serum gamma globulin.‡ The degree of abnormality of both is inhibited by adequate levels of serum albumin or a related protein fraction.§ This is particularly true in the case of cephalin flocculation and less clearly true in the case of thymol turbidity. The abnormality of thymol turbidity has also been shown to be related to the levels of the plasma phospholipids.‖ Insufficient electrophoretic data were available for appraisal of the role of the changes in the plasma proteins in the development of abnormality of the thymol-turbidity and cephalin-flocculation tests.

The importance of leucopenia in the diagnosis of lupus erythematosus is traditional. It is, therefore, of interest that leucopenia could be demonstrated in only 60% of the patients in this group. In many of these patients the leucocyte count had to be estimated on a number of occasions to demonstrate levels below 5000. This is similar to the experience of Michael and co-workers.[39] In contrast to this traditionally important laboratory feature were the results with the thymol-turbidity test, which yielded abnormal results in all patients studied. Thus, it seems that as a nonspecific laboratory index of lupus erythematosus abnormality of the thymol turbidity is more consistent than significant depression of the peripheral leucocyte count.

The frequency of hyperbilirubinemia, with the infrequency of the elevation of the level of direct-reacting bilirubin, suggests that the hyperbilirubinemia was usually hemolytic, in agreement with the reported frequency of hemolytic anemia in this disease.¶ In three patients this was confirmed by demonstrating a direct positive Coombs test. In this group also were seen "biological false positive" results with the serologic test for syphilis. This has been described in a number of other studies as well.# In several of these patients,

* References 12-19.

† References 32-37.

‡ References 34-37.

§ References 34-37.

‖ References 35-38.

¶ References 40 and 41.

References 12-15, 42, and 43.

in keeping with other [44] reports, cryoglobulins and/or cold agglutinins were noted in the serum.

SUMMARY AND CONCLUSIONS

In 25 patients with lupus erythematosus hepatic function was studied. In all the thymol turbidity was found to be abnormal at some time during the course of the disease. Abnormality of the sulfobromophthalein (Bromsulphalein) excretion was less frequent and was usually of slight degree. The cephalin-flocculation test was abnormal more frequently than the sulfobromophthalein test and less frequently than the thymol-turbidity levels. Hyperbilirubinemia, largely of an indirect variety, was found in one-fourth of the patients. The results of these studies, as well as evidence obtained by liver biopsy and at autopsy, led to the conclusions that the apparent hepatic dysfunction represented, in most patients, abnormal plasma protein patterns rather than actual hepatic parenchyma abnormality. In four patients actual hepatic abnormality seemed present. Other abnormal laboratory phenomena observed included the demonstration of "biological false positive" serological tests for syphilis, cryoglobulins, cold agglutinins, and a positive Coombs test in several patients.

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Therapeutic Agents in Rheumatic Carditis

Comparative Effects of Acetylsalicylic Acid, Corticotropin, and Cortisone

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Although the symptoms of acute rheumatic fever appear to be favorably altered by a number of drugs,* the essential problems of therapy are to prevent death from acute carditis and to decrease the incidence of subsequent valvular heart disease.[4] The present report compares the effect of acetylsalicylic acid (Aspirin), cortisone, and corticotropin (ACTH) on valvular heart disease as evidenced by the presence of murmurs 14 months after the onset of therapy.† A previous paper [5] concerning the same group of patients dealt with a comparison of the effects of these drugs on the acute course of rheumatic fever.

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* References 1-3.

† Dr. David Rutstein, Chairman, Miss Marjorie Bellows, Statistician, and the principal investigators of the Cooperative Rheumatic Fever Study gave advice and the protocol used in this study.

METHODS

Details of the methods employed and the population involved have been described elsewhere.[5] All male airmen admitted to Warren Air Force Base Hospital who met the standard diagnostic criteria and who exhibited signs of rheumatic activity on the day treatment began were accepted for study. Each patient was assigned a number in consecutive fashion, and the selection of the form of therapy was predetermined in a random fashion. The dosage schedule employed is presented in Table 1.‡

TABLE 1.—*Schedules of Treatment*

Drug	Daily Dose, Mg.	Day of Treatment
Acetylsalicylic acid*	60/lb.	1 and 2
	40/lb.	3-7
	30/lb.	8-42
Cortisone†	300	1
	200	2-5
	100	6-21
	75	22-35
	50	36-42
Corticotropin‡	120	1-4
	100	5-7
	80	8-14
	60	15-21
	40	22-35
	20	36-42

* No daily dose exceeded 9 gm. (150 grains). Administered orally every four hours for 48 hours and every six hours thereafter.

† Administered intramuscularly in a single daily dose.

‡ Administered intramuscularly in equally divided doses every six hours.

Therapy was administered for six weeks, but if three weeks after the cessation of treatment any patient still met the established criteria for rheumatic activity retreatment was instituted for four weeks, with the same drug and dosage schedule. All patients received 3 gm. of potassium chloride orally each day during treatment and a diet containing less than 2 gm. of sodium per day for the first four weeks. In order to eradicate streptococci and to prevent new streptococcal infections, each patient received penicillin, after which they were

‡ Corticotropin (ACTHAR) was furnished by The Armour Laboratories, Chicago.

given sulfadiazine (1.0 to 1.5 gm. per day).§ The patients were kept at bed rest for at least nine weeks and were then allowed gradually increasing activity.

Observations were made by one of three observers. The patients were examined at monthly intervals for a total period of 14 months after the onset of treatment. Blood was drawn for determination of erythrocyte sedimentation rate, a complete blood cell count and sulfadiazine and antistreptolysin O determinations. In addition, a urinalysis, electrocardiogram, and throat culture were obtained. At 8 and 14 months a roentgenogram of the chest was obtained. Any patient with clinical or bacteriological signs of a streptococcal respiratory infection during the follow-up period was given penicillin for at least 10 days.

Evaluation of Murmurs.—At the 14-month examination a final evaluation of the cardiac status of all patients was made. Mitral valvular disease was thought to be present when a medium- or high-pitched blowing apical systolic murmur, occupying most of systole, had been heard consistently throughout the follow-up period. Apical middiastolic murmurs were heard only in two patients with persistent apical systolic murmurs and were classified as indicative of mitral valvular disease. Apical systolic murmurs which were not as pronounced as in the preceding group and were heard with less regularity were designated as probable mitral valvular disease. Frequently throughout the follow-up period, however, these murmurs were described as the murmur indicative of mitral valvular disease by one of the observers. The murmurs designated as indicative of aortic valvular disease were high-pitched blowing diastolic murmurs most easily heard at the third left intercostal space near the sternal border. The murmurs listed as indicative of mitral or aortic valvular disease and probable mitral valvular disease were classified as significant murmurs. Those murmurs called nonsignificant were systolic in time, frequently varied with position or respiration, and were located either at the apex or along the left sternal border. Generally, they were not heard consistently throughout the follow-up period and were thought to be functional or of questionable significance. The division of the systolic murmurs into significant and nonsignificant was somewhat arbitrary and was not based on knowledge of the significance of the murmur in relationship to permanent valvular heart disease.

§ During the follow-up period two patients developed a dermatitis attributed to sulfadiazine, and in one patient massive hematuria, which subsequently disappeared, was noted. All three patients then received penicillin for prophylaxis.

An independent observer,‖ who knew nothing of the patient's history, previous or present cardiac findings, or therapy, examined 87% of the patients between the 10th and 16th month after the start of therapy. Complete agreement on the presence or absence of a murmur and its significance was obtained in 79% of the patients. In 16% of the patients there was disagreement concerning the presence or absence of a murmur thought to be nonsignificant by one of the parties. In 2% there was disagreement concerning the significance of a murmur heard by both parties, and in 3% there was disagreement concerning the presence or absence of a murmur thought to be significant by one of the observers. Where disagreements were present on the first examination, almost complete agreement was obtained on reexamination, with changes in findings occurring with the same frequency between the two parties involved.

COMPARABILITY OF TREATMENT GROUPS

Of 152 patients who met the established criteria and were included in the study, 135 were followed for 14 months from the start of treatment. Two acetylsalicylic acid-treated patients and two corticotropin-treated patients who developed toxic manifestations which necessitated discontinuance of therapy were excluded from the analysis. In addition, eight acetylsalicylic acid-treated patients, four cortisone-treated patients, and one corticotropin-treated patient were discharged from military service for medical reasons not directly related to rheumatic fever (i. e., duodenal ulcer) or had completed their tour of duty before the 14th follow-up examination.¶ Thus, 53 patients treated with acetylsalicylic acid, 41 patients treated with cortisone, and 41 patients treated with corticotropin were followed for 14 months from the start of treatment and form the basis of this report.

‖ Dr. Walter Pritchard, Associate Professor of Medicine, Western Reserve University School of Medicine, was the independent observer for this study.

¶ All the patients excluded from the study were followed for at least eight months. Of the eight patients treated with acetylsalicylic acid no murmur was heard in four at the time of discharge. In two patients the murmur indicative of mitral valvular disease was present; in one patient the murmur of probable mitral valvular disease was heard, and in one patient a nonsignificant murmur was heard. Of the four patients treated with cortisone no murmur was heard in three at the time of discharge and in one patient a nonsignificant murmur was present. In the one corticotropin-treated patient who did not complete the follow-up period no murmur was heard at the time of discharge.

TABLE 2.—*Comparison of Treatment Groups at the Time Therapy Was Instituted* *

	Acetyl-salicylic Acid	Corti-sone	Cortico-tropin
Patients, No.	52	38	38
Average day of illness	8.0	8.3	8.3
Median day of illness	6	7	7
Per cent of patients with carditis	11.5	18.4	10.3
1. Development of or change in murmur	7.7	10.5	7.7
2. Change in heart size	0.0	0.0	0.0
3. Pericarditis	1.9	5.3	0.0
4. Congestive heart failure	1.9	2.6	2.6
Per cent of patients with abnormal atrioventricular conduction	26.9	28.9	28.9
Rheumatic fever history, per cent of patients with			
1. Definite history	1.9	5.2	2.6
2. Doubtful history	15.4	18.4	10.5

* Including only patients observed for 14 months from the onset of therapy and patients who did not have preexisting rheumatic heart disease.

The day of illness when treatment was started (Table 2) ranged from 2 to 52 days in the acetylsalicylic acid-treated patients, from 2 to 32 days in the cortisone-treated patients, and from 2 to 28 days in the corticotropin-treated patients. Over 50% of the patients in each group were treated within the first 7 days of the onset of their rheumatic illness; 85%, within 14 days. Major and minor manifestations of rheumatic fever were present with similar frequency among the three groups.

Carditis # was judged to be present at the time therapy was instituted in 12% of the acetylsalicylic acid-treated patients, 18% of the cortisone-treated patients, and 10% of the corticotropin-treated patients. In retrospect, many more patients in each treatment group probably had carditis at the start of treatment, but, since therapy was started early, often allowing only a 24-hour baseline period of observation, the development of or change in an existing murmur frequently took place after therapy had started. Approximately 35% of the patients in each treatment group had abnormal atrioventricular conduction at the start of therapy. Five per cent of the patients treated with cortisone gave a definite past history of rheumatic fever, while 2% and 3% in the acetylsalicylic acid and corticotropin groups, respectively, gave such a history. There was one patient in the acetylsalicylic acid-treated group and three patients in each of the corticotropin- and cortisone-treated groups who were thought to have preexisting rheumatic heart disease and are excluded from the analysis and discussed separately. Further details concerning the comparability of the three treatment groups have been presented previously.[5]

Development of or change in murmur, change in heart size, pericarditis, or congestive heart failure; not including patients with abnormal atrioventricular conduction as the sole manifestation of carditis.

TABLE 3.—*Occurrence of Murmurs Fourteen Months After Onset of Treatment* *

Classification of Murmur	Acetylsalicylic Acid (52 Patients)		Cortisone (38 Patients)		Corticotropin (38 Patients)	
	No.	%	No.	%	No.	%
Patients with significant murmurs†	18	34.6	7	18.4	13	34.2
Mitral valvular disease	10	19.2	3	7.9	6	15.8
Mitral and aortic valvular disease	4	7.7	0	0.0	1	2.6
Probable mitral valvular disease	4	7.7	4	10.5	6	15.8
Patients with nonsignificant murmurs	8	15.4	8	21.1	6	15.8
Total patients with any murmur	26	50.0	15	39.5	19	50.0
Total patients without a murmur	26	50.0	23	60.5	19	50.0

* Excluding one patient in the acetylsalicylic acid group and three each in the cortisone and corticotropin groups who had rheumatic heart disease on admission.
† Note definition under "Methods."

RESULTS

In those patients who completed the period of observation (Table 3) a murmur was heard 14 months after the onset of therapy in 50% of the patients in the corticotropin- and acetylsalicylic acid-treated groups, in contrast with 40% of those patients treated with cortisone. Murmurs classified as being significant were heard at 14 months in approximately 34% of the patients in each of the acetylsalicylic acid- and corticotropin-treated groups, in contrast with 18% in the cortisone-treated group. There were four patients in the acetylsalicylic acid-treated group and one in the corticotropin-treated group in whom the murmurs of both mitral and aortic valvular disease were present. In no patient in the cortisone-treated group were the two murmurs present at this time.

The observed differences in the presence or absence of any murmur between the cortisone- and corticotropin- and acetylsalicylic acid-treated groups were not great. However, if only those murmurs classified as significant are considered, the difference approaches statistical significance (p=0.11) and may indicate that cortisone therapy has exerted an effect.

Apical Systolic Murmurs

If cortisone therapy of acute rheumatic fever is exerting a beneficial effect on valvulitis, then certain changes should be demonstrable during the period that the hormone is actually being administered. The period of observation following cessation of therapy is also important, because of the frequent occurrence of signs of rheumatic activity immediately following withdrawal of treatment and because streptococcal infections and recurrent attacks of rheumatic fever may alter the cardiac status at the final evaluation at 14 months. An analysis of the apical systolic murmurs considered significant was therefore made, in order to determine where in the period of observation the possible favorable effects of cortisone became apparent.

Relationship of Cardiac Status at Start of Therapy to Occurrence of Significant Systolic Murmurs at Fourteen Months (Table 4).—At the time of onset of treatment there was little difference among the three groups in the presence of murmurs (38.5%, 36.8%, and 42.1%). Among patients in whom no murmur was detected at the start of treatment, a significant apical systolic murmur was heard at 14 months in 13% of those patients treated with cortisone, in contrast with 25% and 27% of those patients treated with acetylsalicylic acid and corticotropin, respectively. Among patients with a murmur at the start of therapy, a significant apical systolic murmur was present at 14 months in 29% of the patients treated with cortisone, in contrast with 50% of the acetylsalicylic acid-treated patients and 44% of the corticotropin-treated patients. Most patients with murmurs at 14 months came from the group who had murmurs at the start of therapy, and the incidence of murmurs at 14 months in those patients without a murmur at the start of therapy was one-half that of the other group. The effect of cortisone in contrast with that of corticotropin and acetylsalicylic acid was apparent in each group. It should be emphasized that the majority of patients in both groups were treated early in the course of the illness, and the findings may not reflect what might be observed in civilian institutions.

Immediate Effect of Therapy on Murmurs.—The effect of therapy on the appearance of murmurs in patients who had no murmurs at the start of treatment is shown in Figure 1. Among the 32 acetylsalicylic acid-treated patients, 18 developed an apical systolic murmur during therapy, and in six of these patients a significant murmur was

Table 4.—*Occurrence of Significant Apical Systolic Murmurs Fourteen Months After Start of Therapy According to Presence or Absence of Murmur at Start of Therapy* *

Cardiac Status at Start of Therapy	Acetylsalicylic Acid (52 Patients)			Cortisone (38 Patients)			Corticotropin (38 Patients)			Total (128 Patients)		
	Patients, No.	Per Cent of Total No. of Patients	Per Cent with Significant Murmur at 14 Mo.	Patients, No.	Per Cent of Total No. of Patients	Per Cent with Significant Murmur at 14 Mo.	Patients, No.	Per Cent of Total No. of Patients	Per Cent with Significant Murmur at 14 Mo.	Patients, No.	Per Cent of Total No. of Patients	Per Cent with Significant Murmur at 14 Mo.
Murmur present†	20	38.5	50.0	14	36.8	28.6	16	42.1	43.8	50	39.1	42.0
No murmur present	32	61.5	25.0	24	63.2	12.5	22	57.9	27.3	78	60.9	21.8

* Excluding one patient in the acetylsalicylic acid group and three each in the cortisone and corticotropin groups who had rheumatic heart disease on admission.

† The murmur must have been present for four days out of seven consecutive days and thought to be significant on at least one occasion during the seven days.

heard 14 months later. Fifteen of the 18 new murmurs appearing during therapy were still present at the end of therapy. Of the 24 patients treated with cortisone, 5 patients developed an apical systolic murmur during therapy, but in none of these 5 patients was a significant apical systolic murmur heard at 14 months. Three of the five new murmurs appearing during therapy were still present at the end of therapy. Among the 22 corticotropin-treated patients in whom no murmurs were heard at the start of therapy, 12 patients developed an apical systolic murmur during therapy, and in 5 of these patients a significant apical systolic murmur was heard 14 months later. Seven of the 12 new murmurs appearing during therapy were still present at the end of therapy. The observed difference in the appearance of new murmurs during therapy between the cortisone- and acetylsalicylic acid- and corticotropin-treated patients was significant (p=0.009).

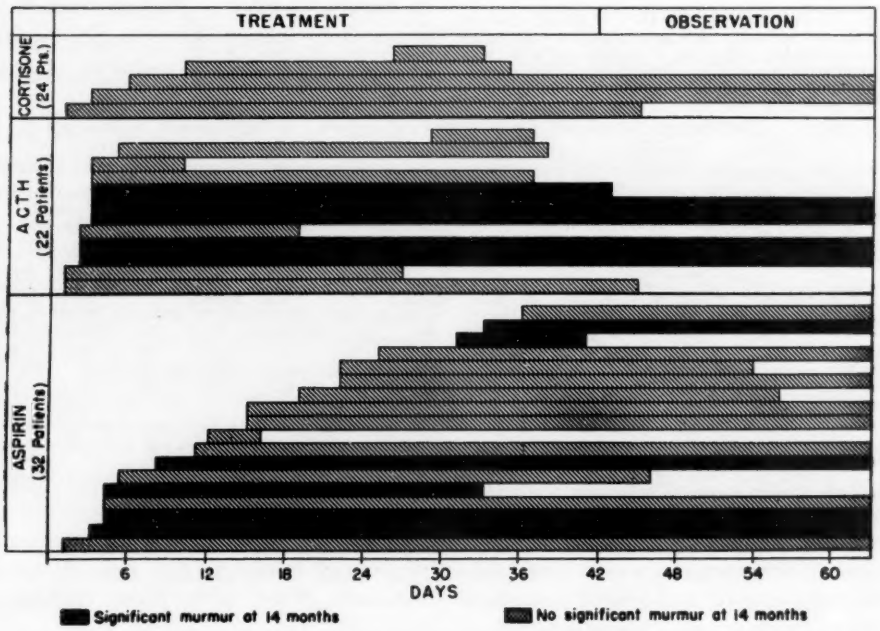

Fig. 1.—Time of appearance of apical systolic murmurs during therapy in patients with no murmur at the start of treatment.

Figure 1 shows when these systolic murmurs became apparent. In the acetylsalicylic acid-treated patients the time of appearance of the murmurs is fairly evenly distributed throughout the treatment period. In the corticotropin-treated group almost all the murmurs appeared within the first five days of treatment, and in the cortisone-treated group, within the first 10 days of therapy. The rather prompt appearance of apical systolic murmurs shortly after the start of therapy in the hormone-treated patients, especially those receiving corticotropin, may, perhaps in part, be related to the earlier appearance of bradycardia in these groups and to the hypervolemia present at this time.

The effect of therapy on the disappearance of murmurs in patients who had a murmur at the start of therapy is shown in Figure 2. Only those patients in whom the murmur disappeared during the treatment period or during the following three weeks of observation are considered. There was only one patient in the acetylsalicylic acid-treated group in whom a murmur was heard at the start of therapy and whose murmur dis-

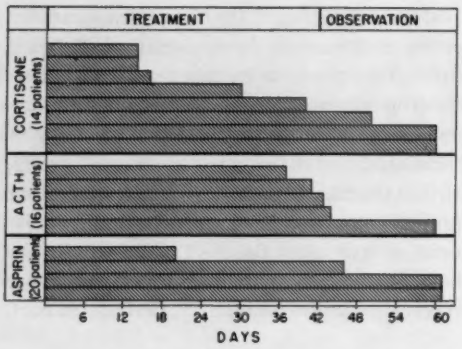

Fig. 2.—Time of disappearance of apical systolic murmurs in patients with a murmur at the start of therapy which disappeared during the first nine weeks.

and the greatest number of murmurs disappeared during the treatment period. It seems possible that this observed effect was caused by a favorable influence of cortisone. It also might indicate that treatment with larger doses of cortisone for a longer period of time in this group of patients might have been beneficial.*

Alterations in Cardiac Status Following Cessation of Therapy and Their Relationship to Streptococcal Infections and New Attacks of Rheumatic Fever.—An important factor in evaluating therapy is the change in cardiac findings noted after the cessation of treatment and the correlation of streptococcal respiratory infections and new attacks of rheumatic fever with these changes in murmurs.

TABLE 5.—*Occurrence of Significant Apical Systolic Murmurs Fourteen Months After Start of Therapy According to Presence or Absence of Murmur at End of Therapy* *

Cardiac Status at End of Therapy	Acetylsalicylic Acid (52 Patients)			Cortisone (38 Patients)			Corticotropin (38 Patients)			Total (128 Patients)		
	Patients, No.	Per Cent of Total No. of Patients	Per Cent with Significant Murmur at 14 Mo.	Patients, No.	Per Cent of Total No. of Patients	Per Cent with Significant Murmur at 14 Mo.	Patients, No.	Per Cent of Total No. of Patients	Per Cent with Significant Murmur at 14 Mo.	Patients, No.	Per Cent of Total No. of Patients	Per Cent with Significant Murmur at 14 Mo.
Murmur present†	34	65.4	41.1	12	31.6	33.3	21	55.3	57.1	67	52.3	44.7
No murmur present	18	34.6	22.2	26	68.4	11.5	17	44.7	5.9	61	47.7	13.1

* Excluding one patient in the acetylsalicylic acid group and three each in the cortisone and corticotropin groups who had rheumatic heart disease on admission.

† The murmur must have been present for four days out of seven consecutive days and thought to be significant on at least one occasion during the seven days.

appeared during therapy. Shortly after the cessation of treatment there were three additional acetylsalicylic acid-treated patients in whom murmurs were no longer detected. In two patients in the corticotropin-treated group a murmur heard at the start of treatment disappeared during therapy, and shortly after the cessation of treatment there were three additional patients in whom a murmur was no longer heard. In one patient in the corticotropin-treated group the murmur reappeared and was called a significant apical systolic murmur at the 14th month. In five cortisone-treated patients murmurs heard at the start of therapy disappeared during therapy, and shortly thereafter a murmur present at the start of treatment was no longer detected in three additional patients.

Thus, among those patients receiving cortisone the fewest new murmurs appeared

From Table 5 it can be seen that 32% of the cortisone-treated patients had an apical systolic murmur at the end of treatment, in contrast with 65% of the acetylsalicylic acid-treated patients and 52% of the corticotropin-treated group. In those patients in whom a murmur was heard at the end of therapy the fewest patients with a significant apical systolic murmur at 14 months occurred in the cortisone-treated group. In those patients with no murmur at the end of therapy the number in each treatment group developing significant systolic murmurs at 14 months

* Catanzaro, F., and Stolzer, B. L.: To be published.

was too small for comparison. When all treatment groups are combined, those patients having a murmur at the end of therapy were approximately three times as likely to have a murmur at the 14th-month follow-up as the group without a murmur at the end of therapy.

Approximately 20% of the patients in each treatment group continued to exhibit isolated signs of rheumatic activity at the time of the first monthly examination. One patient in the corticotropin-treated group and three patients in the acetylsalicylic acid-treated group exhibited signs of continued rheumatic activity for the 14 months. Two of the three acetylsalicylic acid-treated patients had definite evidence of increasing cardiac damage during this period. One additional patient in the acetylsalicylic acid-treated group, who was followed for only six months, had signs of continued rheumatic activity and increasing cardiac damage. No patient in the cortisone-treated group exhibited evidence of continued rheumatic activity. Activity of the rheumatic process was severe enough to warrant a second course of therapy in seven acetylsalicylic acid-treated patients, one corticotropin-treated patient, and one cortisone-treated patient. With few exceptions, the second courses of therapy were started three to five weeks after the cessation of the initial treatment.

During the follow-up period there were 14 patients (11%) who developed streptococcal pharyngitis, as evidenced by symptoms or signs of a sore throat, a positive culture for typable Group A streptococci, and an increased antistreptolysin titer. Eight were among the cortisone-treated patients; five, among the corticotropin-treated patients, and one, among the acetylsalicylic acid-treated patients. There were four additional patients in the cortisone-treated group, three in the corticotropin-treated group, and one in the acetylsalicylic acid-treated group who transiently acquired a typable Group A Streptococcus on throat culture but were asymptomatic and did not develop an antistreptolysin response. The streptococcal infections, with one exception, were promptly treated with penicillin. One patient in the cortisone-treated group received penicillin 10 to 14 days after the onset of the streptococcal pharyngitis and subsequently developed signs of a new attack of rheumatic fever. No murmur was detected at 14 months in this patient. In the remaining patients who had streptococcal infections or transiently acquired streptococci, there was no evidence of renewed rheumatic activity and no evidence of any change in or development of a murmur. Thus, the fact that the fewest significant murmurs heard at 14 months occurred in the cortisone-treated group cannot be correlated with differences in the incidence of streptococcal respiratory infections and new attacks of rheumatic fever during the follow-up period. Actually the cortisone-treated patients had the greatest possibility for recurrence of activity and the development of new murmurs.

Aortic Diastolic Murmurs

During treatment aortic diastolic murmurs were heard in eight patients: three in the acetylsalicylic acid-treated group, four in the corticotropin-treated group, and one in the cortisone-treated group. Two of the murmurs in the acetylsalicylic acid-treated group and one in the corticotropin-treated group were heard throughout the 14 months of observation; the remainder disappeared. Two additional patients in the acetylsalicylic acid-treated group developed aortic diastolic murmurs after the completion of therapy. In one of these patients there was continued rheumatic activity, but neither had evidence of a streptococcal infection or a new attack of rheumatic fever. Thus, at the 14th-month follow-up an aortic diastolic murmur was heard in four patients treated with acetylsalicylic acid, in one patient treated with corticotropin, and in no patient treated with cortisone.

Mitral Middiastolic Murmurs

During therapy apical middiastolic murmurs were heard in 10 patients: 4 in the acetylsalicylic acid-treated group, 5 in the

corticotropin-treated group, and 1 in the cortisone-treated group. In addition, in three acetylsalicylic acid-treated patients and one corticotropin-treated patient apical middiastolic murmurs were heard transiently throughout the follow-up period. Except for three patients in the acetylsalicylic acid-treated group, all murmurs disappeared. The fewest apical middiastolic murmurs occurred in the cortisone-treated group, and none persisted for 14 months.

Effect of Rebound on Murmurs

The majority of patients in each treatment group exhibited signs of clinical or laboratory rebound which did not persist throughout the 14 months. The extent to which the rebound phenomenon influenced existing murmurs could not be determined.

Evaluation of Therapy in Patients with Preexisting Rheumatic Heart Disease

The appearance of new murmurs in patients who had rheumatic heart disease at the onset of therapy is shown in Table 6. Aortic diastolic murmurs appeared in three patients, one in each of the treatment groups. The evaluation of the appearance of new murmurs in patients with preceding rheumatic heart disease was difficult, and the number of patients with rheumatic heart disease in this study too small to note any trend. However, significant murmurs were heard for the first time in patients in each of the treatment groups.

Table 6.—*Appearance of New Murmurs in Patients Who Had Rheumatic Heart Disease at Onset of Therapy*

	History of Previous Rheumatic Fever	Cardiac Diagnosis on Admission*	New Murmur	First Appeared	Final Cardiac Diagnosis
Acetylsalicylic acid					
Patient 1.....	5 yr. before 10 yr. before 14 yr. before	M. I., M. S.	Aortic diastolic	5th day of therapy	M. I., M. S., A. I.
Cortisone					
Patient 1.....	7 yr. before	M. S.	Mitral systolic	3d day of therapy	M. I., M. S.
Patient 2.....	3 yr. before	M. I., M. S., A. I., A. S.	.....	.....	M. I., M. S., A. I., A. S.
			Mitral presystolic	40th day of therapy	
Patient 3.....	None	M. I.	Aortic diastolic	6 mo. after start of therapy	M. I., M. S., A. I.
Corticotropin					
Patient 1.....	5 yr. before 10 yr. before 15 yr. before	M. I., A. I.	.....	Aortic diastolic disappeared on 22d day of therapy	M. I.
Patient 2.....	None	M. I., M. S.	Aortic diastolic	28th day of therapy	M. I., M. S., A. I.
Patient 3.....	8 yr. before	M. I.	.....	.....	M. I.

* M. I., mitral insufficiency; M. S., mitral stenosis; A. I., aortic insufficiency; A. S., aortic stenosis.

reactivation after the cessation of therapy. During the rebound eight new murmurs appeared, of which five persisted for 14 months. Two were aortic diastolic murmurs in patients treated with acetylsalicylic acid who also had significant apical systolic murmurs. Three were mitral systolic murmurs, two in the cortisone-treated group and one in the acetylsalicylic acid-treated group. In the two patients in the cortisone-treated group murmurs had been heard previously during the treatment period but were inconstant at the time and were not considered to be significant. There were three additional patients who developed murmurs during the

Effect of Therapy on Other Manifestations of Carditis

Approximately 35% of the patients in each treatment group had abnormal atrioventricular conduction at the start of therapy.[5] Abnormal P-R intervals appeared for the first time after the start of treatment with the same frequency in each group. Corticotropin and cortisone appeared to shorten the

length of time a P-R interval remained abnormal when compared with the effect of acetylsalicylic acid. There were too few patients (6%) with abnormal atrioventricular conduction as the sole manifestation of carditis to determine its relationship to the presence of significant murmurs at 14 months. However, when the total number of patients is considered, regardless of other signs of carditis, significant murmurs were present at 14 months in 22% of those patients who never had abnormal atrioventricular conduction at any time during the period of observation, in contrast to 38% of those patients who had abnormalities in atrioventricular conduction before, during, or immediately after treatment.

Chest roentgenograms taken at weekly intervals during treatment revealed slight differences in the size of the heart, which appeared to be directly related to the discontinuance of the low-salt diet after the fourth week followed by an increased gain in weight in the hormone-treated group at this time.[5] At the end of nine weeks of observation, the average heart size in all three treatment groups was essentially equal to that at the onset of treatment. Chest roentgenograms obtained at the 8th- and 14th-month follow-up revealed definite cardiac enlargement in only one patient (from the acetylsalicylic acid-treated group) who did not have preexisting rheumatic heart disease.

There were three patients, one in each treatment group, with signs of mild cardiac failure at the start of therapy.[5] The signs of congestive heart failure had disappeared within seven days in all three patients. After therapy was discontinued, congestive heart failure occurred in two other patients, both from the acetylsalicylic acid-treated group. One patient had preexisting rheumatic heart disease at the time of entry into the study. The other patient developed murmurs indicative of mitral and aortic valvular disease and an enlarged heart during the 14 months.

Pericarditis was present at the start of therapy in two patients treated with cortisone and in one acetylsalicylic acid-treated patient. The pericardial friction rubs disappeared within six days in these patients. Two other patients in the cortisone-treated group, one in the corticotropin-treated group, and two in the acetylsalicylic acid-treated group developed pericardial friction rubs after the cessation of treatment. In two of these patients, one each from the cortisone- and acetylsalicylic acid-treated groups, an apical systolic murmur appeared at this time and was heard consistently for 14 months.

COMMENT

The introduction of therapy early in the course of the illness, before heart lesions have developed, the use of a population with no antecedent heart disease, and the maintenance of prophylaxis against streptococcal infections are of importance in the long-term evaluation of any form of therapy in the treatment of rheumatic fever. In the present study the populations employed were young adult males, the majority of whom were experiencing their first attack of acute rheumatic fever. Treatment was started early in the course of their illness, over 50% of the patients having been treated in the first week. Prophylaxis against streptococcal infections was maintained throughout the observation period, and bacteriological and serological data were obtained to confirm this.

The comparative effects of these drugs on the acute manifestations of rheumatic fever in these patients have been presented elsewhere.[5] Briefly, acetylsalicylic acid afforded the most prompt relief of fever and joint manifestations; cortisone was somewhat less effective. Corticotropin had the most marked effect on the elevated erythrocyte sedimentation rate. Cortisone and corticotropin shortened the duration of abnormal atrioventricular conduction in comparison with acetylsalicylic acid therapy. Signs of a clinical or laboratory reactivation occurred in almost all the patients in each group after the discontinuance of treatment. No one drug employed exhibited marked advantages over the others in the treatment of the acute manifestations of rheumatic fever.

The goal in the treatment of rheumatic fever is to prevent the development of valv-

ular heart disease. Acetylsalicylic acid has been used for many years, with conflicting reports as to its effect on carditis and the prevention of valvular heart disease.† Recently, the same controversy has arisen regarding the effects of corticotropin and cortisone.‡ In the present study there were patients in all three treatment groups who developed murmurs indicative of valvular disease during the 14 months of observation. Thus, neither acetylsalicylic acid nor cortisone nor corticotropin prevented valvular damage in all instances. In the cortisone-treated group there were the fewest patients with significant murmurs as measured by examination at 14 months. Most of the murmurs heard were apical systolic murmurs. Analysis of the apical systolic murmurs was made to see where cortisone was exerting its seemingly beneficial effect. The majority of patients were treated early in the course of the illness, and in those patients treated before the appearance of signs of cardiac damage (those with no murmur at the start of therapy) cortisone prevented the development of apical systolic murmurs to a greater degree than did corticotropin or acetylsalicylic acid. Cortisone also appeared to have a greater effect on the disappearance of murmurs present at the start of therapy. There were fewer patients in the cortisone-treated group who had a murmur at the end of therapy than in the other treatment groups. Among those patients who had a murmur at the end of therapy, the fewest in whom a significant apical systolic murmur was still heard at 14 months were found in the cortisone-treated group. The number of murmurs appearing for the first time after the cessation of therapy was small, with little difference among the three treatment groups. The presence of streptococcal respiratory infections in 11% of the patients and the occurrence of a new attack of rheumatic fever in one patient did not appear to be a factor in the observed differences in the presence of significant apical systolic murmurs at 14 months. Thus, this difference can be explained by the evidence that cortisone seemed to prevent the persistence of murmurs already present at the start of therapy and to prevent the appearance of significant murmurs during the treatment period and to prevent murmurs, to a lesser degree, after the cessation of treatment more than did acetylsalicylic acid or corticotropin. The observed difference at 14 months between the cortisone- and corticotropin-treated patients may be related to the appearance of five apical systolic murmurs within the first five days of corticotropin therapy which were present 14 months later. The possibility still remains that despite random selection of patients for each therapy group the fewest patients with severe carditis were in the cortisone-treated group and that, hence, this group would be expected to have the least number of significant murmurs at 14 months.

Although the numbers are small, aortic valvular disease was commoner in the acetylsalicylic acid-treated patients. In no patient treated with cortisone was an aortic diastolic murmur heard at 14 months. A similar trend was evident with apical middiastolic murmurs. The three patients with persistent apical middiastolic murmurs were in the acetylsalicylic acid-treated group.

Many of the apical systolic murmurs heard during therapy subsquently disappeared and were probably related to the acute illness or myocarditis rather than to specific valvular damage. A small number of these patients may go on to develop valvular heart disease in later years.[4] The division of the murmurs heard at 14 months into significant and nonsignificant was arbitrary, and only the future course of these patients can determine the true significance of these murmurs in regard to permanent heart damage.

In general, most of the patients in all three treatment groups experiencing their first attack of rheumatic fever had little evidence of organic heart disease at the end of 14 months. The lesions were predominantly those of slight mitral valvular disease (in-

† References 3 and 6-8.

‡ References 9-13.

sufficiency), without evidence of other valvular involvement. These patients did exceedingly well, since only four continued to exhibit signs of rheumatic activity. In only one instance, in a patient entering the study without preexisting rheumatic heart disease, was congestive heart failure present during the follow-up period.

The prophylactic regimen instituted, using sulfadiazine, was successful in an area highly endemic for streptococcal respiratory diseases. Eleven per cent of the patients developed streptococcal pharyngitis during the 14-month period of observation. The majority of the infections were not due to failure of sulfadiazine, since these patients had omitted prophylaxis at the time of infection. In these patients prompt treatment of the streptococcal pharyngitis with penicillin for 10 days resulted in no new attacks of rheumatic fever. One patient did develop a new attack of rheumatic fever following streptococcal pharyngitis, but the onset of penicillin therapy had been delayed for from 10 to 14 days.

The basis of this report was a period of 14 months' observation following the onset of treatment for acute rheumatic fever. This is a small fraction of time in the life history and evolution of rheumatic valvular heart disease, but it was felt that some insight into the relative effectiveness of these drugs in preventing valvular damage could be obtained. If cortisone or corticotropin or both are effective in preventing valvular heart disease, then their observed effects are not marked. However, the seemingly favorable effect of cortisone in this study should prompt further investigation.§ Only long-term follow-up studies maintaining the patients on sulfadiazine or antibiotic prophylaxis against streptococcal respiratory infections will give a more definite conclusion as to the relative effectiveness of acetylsalicylic acid, cortisone, and corticotropin in preventing valvular heart disease. Such studies are now under way in a number of civilian hospitals as part of the Cooperative Rheumatic Fever Study of the American Council on Rheumatic Fever.

§ Catanzaro, F., and Stolzer, B. L.: To be published.

SUMMARY

The comparative effects of acetylsalicylic acid, cortisone, and corticotropin on rheumatic carditis, as evidenced by the presence of murmurs during a 14-month period of observation, were observed in 52 patients treated with acetylsalicylic acid, 38 patients treated with cortisone, and 38 patients treated with corticotropin.

The fewest patients with significant murmurs at 14 months occurred in the cortisone-treated group; 18%, in contrast to 34% in those patients treated with either acetylsalicylic acid or corticotropin. It seemed that cortisone prevented the appearance of apical systolic murmurs and caused the disappearance of existing apical systolic murmurs during the treatment period to a greater degree than either acetylsalicylic acid or corticotropin. This difference during therapy was reflected at the 14th-month evaluation. The seemingly favorable effect of cortisone was also evident when aortic diastolic and apical middiastolic murmurs were considered, although the numbers were small.

The differences observed among the three treatment groups do not allow a definite conclusion as to whether cortisone and corticotropin are superior to acetylsalicylic acid in the prevention of rheumatic valvular heart damage.

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Fulminant Normocholesteremic Xanthomatosis (Histiocytosis X)

Report of Incidence in an Aged Woman

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Advancing medical knowledge has not infrequently changed our interpretation of clinical syndromes and diseases. On one hand, well-defined symptom complexes, once thought to represent specific diseases, were found to be caused by more than one pathological process and, conversely, different clinical syndromes were revealed as different phases and manifestations of one and the same nosologic entity. Thus, conditions as different in their clinical manifestations as Hand-Schüller-Christian disease, Letterer-Siwe disease, and eosinophilic granuloma of bone, each once described as disease entities, are now thought by many to represent merely the acute or chronic and the localized or disseminated forms of the same basic pathologic process.*

This change in concept came about with the better understanding of the morphologic picture of these conditions but also with the ever-increasing number of instances in which features of more than one of these diseases occurred simultaneously or subsequently in the same patient.

Hand-Schüller-Christian disease seemed originally to be a well-defined entity characterized by the triad of exophthalmos, polyuria, and granulomatous lesions of the skull. Hand, who in 1893 described the very first case, believed that it was a chronic infection, probably of tuberculous nature.[6] Schüller,[7] in 1915, and Christian,[8] in 1920, related the polyuria to the pituitary gland and considered the disease an endocrine disturbance caused by dyspituitarism. Through Rowland's classic morphologic studies in 1926,[9] it became evident that the disease was the manifestation of a systemic disorder characterized by granulomatous lesions consisting of reticulum cells, histiocytes, cholesterol-containing foam cells, and inflammatory elements, as eosinophiles, polymorphonuclear leucocytes, and round cells. Accordingly, Hand-Schüller-Christian disease was considered to be a disturbance of fat metabolism and was classified with the larger group of the lipoidoses. It became evident that the craniohypophyseal localization indicated by the triad was but one expression of a pathological process which could be protean in its manifestations, since practically all organs could be involved. In the subsequent years, many excellent accounts with detailed descriptions of the clinical and pathological manifestations were published; some of them attempted classifications according to the localization of the lesions.† All authors agreed that the disease was confined to children and young adults and that it was a slowly progressing chronic process, being fatal mainly as the result of complications or of involvement of vital organs.

In 1933 and 1934, Siwe [17] and Letterer [18] described independently a new disease of infants and young children which was manifested by hepatosplenomegaly and generalized lymphadenopathy, but without changes in the white blood cell picture, and which seemed to be acute, rapidly progressing, and fatal in most instances. Histological studies

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* References 1-5.

† References 10-16.

of the lesions revealed proliferation of monocytic histiocytes, with accumulation of eosinophiles and the appearance of giant cells, which in some instances showed a foamy cytoplasm due to intracellular lipid deposition. The disease, although usually referred to as Letterer-Siwe disease, was designated by the original observers as "reticuloendotheliosis" and "xanthomatous lymphogranulomatosis," respectively. Subsequently, several authors pointed out the similarity as well as the variability of the pathologic changes encountered in this condition and in Hand-Schüller-Christian disease.‡ Although age incidence, organ involvement, and clinical course differed significantly, the morphologic changes suggested a nosologic relationship. In 1940, Lichtenstein and Jaffe [23] described the eosinophilic granuloma of bone. As a localized and usually harmless self-limiting disease of infants and young children, it had clinically nothing in common with the two other syndromes but showed a histologic similarity with some of the lesions.

All three conditions are characterized by the presence of a granulomatous process. While in eosinophilic granuloma of bone the lesion consists primarily of eosinophiles and monocytes, it is composed in Letterer-Siwe disease mostly of monocytes and reticulum cells with occasional giant cells and eosinophiles. In Hand-Schüller-Christian disease the granulomatous process discloses a more pleomorphic picture, consisting of reticulum cells, histiocytes, lipophages, eosinophiles, multinucleated giant cells, lymphocytes, and fibroblasts. The functional relationship of these three conditions which was thus suggested seemed indicated further by the observations of coexistence of lesions of various types in the same patient and in the same organs.§ It soon appeared that in eosinophilic granuloma the manifestations of the disease were localized and confined to the bone, while in the generalized form the same process would develop either into the acute Letterer-Siwe type, with hepatosplenomegaly, lymphadenopathy, and involvement of other parenchymatous organs, or into the chronic type, with the features of Hand-Schüller-Christian disease. The differences in age incidence and prognosis seemed also explainable on the basis of acuity and chronicity of the pathological process. When developing in early life, the disease appeared in its acute, fulminant form, while the chronic form seemed to be compatible with survival and was thus found also in early adulthood and occasionally in the adult.

Thannhauser [3] was among the first to propose a unified concept of the disease. To emphasize its three specific morphological features, he called it "eosinophilic xanthomatous granuloma." Moreover, he suggested a new pathological mechanism and believed that the xanthomatous granulomas of the chronic phase (Hand-Schüller-Christian) were the result of an intracellular enzymatic imbalance in the histiocytes and reticulum cells, which were transformed to cholesterol-containing foam cells. The absence of any hypercholesterolemia, which should be expected in a general disturbance of cholesterol metabolism, became thus understandable. Lichtenstein,[4] though in agreement with Thannhauser's concept, objected to the new name on morphologic grounds, since it did not apply accurately to the component lesions of the disease. While the eosinophilic granuloma of bone may never show an accumulation of cholesterol, the lesions of Hand-Schüller-Christian disease, on the other hand, may be void of eosinophiles. He emphasized that the common factor in all three component conditions is a histiocytosis which may or may not lead to secondary lipidization of these cells, depending on severity and duration of the process, and he proposed the name "histiocytosis X" to indicate the unity of the disease complex and also to express the lack of our knowledge as to its cause and development.

Recently we had the opportunity to observe an instance of fulminant normocholesterolemic xanthomatosis in a 67-year-old woman. This case is presented not only because it seems to be the oldest patient on record with this disease, but also because it

‡ References 19-22.

§ References 24-27.

provides an example of this nosologic entity and demonstrates its diversified clinical and morphologic picture.

REPORT OF A CASE

Patient B. W., K1152, a 67-year-old white woman, was admitted to the Jewish Chronic Disease Hospital of Brooklyn on Jan. 15, 1954. She gave a six-month history of progressive weakness, marked weight loss, and pain in her hip joints and legs. The patient was a retired school teacher, alert, intelligent, and cooperative. She stated that she had been entirely well until June, 1953. At that time she had as a matter of routine an annual physical check-up and had been found to be in good physical shape. Since one of her siblings had died of diabetes several years ago, a determination of the blood sugar and test for urine sugar were done regularly. Both were reported as normal. Apparently the only nonphysiological signs of long standing were large xanthomata bilaterally on the eyelids and vitiliginous areas over the trunk and both upper extremities, which were said to have been present for at least 15 years. Shortly after this examination she noticed a sudden and rapid loss of weight in spite of good appetite and adequate food intake. She began to develop episodes of chills and fever, which recurred until the date of admission. A maculopapular eruption appeared over her entire body and persisted from that time on. She also developed severe pyorrhea and lost most of her teeth within a period of six weeks. When she began to complain of increasing thirst, diabetes mellitus was suspected. She was hospitalized for three weeks in July, 1953. The diagnosis of diabetes mellitus was established, but no other cause for her rapidly progressing debilitation was found. While in this first hospital she had an episode of severe pain in the right leg; a roentgenologic study of this leg was negative, and the pain subsided spontaneously within a few days. X-ray examinations of the chest and the gastrointestinal tract were negative. Her diabetes was easily controlled with dietary restrictions and 15 units of protamine zinc insulin daily. This management, however, did not prevent a further downhill course. A new hospitalization for differential diagnostic study was advised, and the patient spent the month of November, 1953, in another hospital. Here her diabetes was found to be out of control, with a fasting blood sugar level of 325 mg. per 100 cc. Because of the bone pain and the recurrent episodes of fever, a skeletal survey including the skull and intravenous pyelography were performed. No bone lesions were found, but hydronephrotic changes in the left kidney were noted. Chest and gastrointestinal x-ray studies were again normal. Other laboratory studies were within normal limits, such as total blood cholesterol (196 mg. per 100 cc.), free cholesterol (63 mg. per 100 cc.) and cholesterol esters (*32%*). Two BMR determinations, however, were markedly elevated: +43 and +47%. The patient was discharged with the diagnosis of diabetes and hyperthyroidism. No therapy was recommended except an increase of insulin.

Physical Examination.—At the time of admission to the Jewish Chronic Disease Hospital of Brooklyn on Jan. 15, 1954, the physical examination showed a cachectic white woman weighing 72 lb. (32.4 kg.), unable to walk without assistance because of extreme weakness. Her skin showed extensive areas of vitiligo over trunk and extremities, as well as a generalized eruption consisting of acuminate hyperkeratotic follicular macules with some diffuse nondescript yellowish pigmentation. Face: bilateral marked xanthelasma of the eyelids. Eyes: cataracts bilaterally, pupils reacting to light and in accommodation. Fundi: negative. Mouth: several small gingival ulcerations which had some yellowish appearance at their margins. Neck: No enlargement of the thyroid gland; numerous peanut-sized painless mobile lymph nodes in the anterior cervical triangle; palpable lymph nodes were present also in both axillary, inguinal, and femoral regions and at the left elbow. Chest: breath sounds decreased throughout the left lung, otherwise negative. Heart: a loud harsh systolic murmur was heard at Erb's point; regular sinus rhythm; pulse rate, 84; B.P., 140/90. Abdomen: Liver and spleen were not palpable. Rectum: an irregular hard tumor was felt, apparently belonging to the uterus, which was in a retroverted position.

Laboratory studies disclosed normal values for calcium, CO_2-combining power, alkaline phosphatase, thymol turbidity, chlorides, and potassium. There was a moderate decrease of sodium; blood sugar was 245 mg. per 100 cc.; cholesterol, 189 mg. per 100 cc. Free cholesterol, 54 mg. per 100 cc.; cholesterol esters, 71% of total; total protein, 4.46 gm. per 100 cc., with an A/G ratio of 0.91. The gamma globulin as determined electrophoretically was normal. The hematologic study disclosed a mild secondary anemia and a white blood cell count of 12,000, with a normal differential count. Urinalysis: S. G., 1.005; albumin, 2+; sugar and blood, negative; numerous WBC in sediment; Bence-Jones proteins were positive twice.

Biopsy of a cervical lymph gland on Jan. 26, 1954, and of the gingiva on Feb. 9, 1954, disclosed each time the picture of a nonlipid histiocytosis. Bone marrow study: myelocytes, 38%; metamyelocytes, 12%; stab cells, 10%; PMN, 35%; eosinophiles, 5%; GE ratio, 80/20. Plasma cells of the mature type were slightly increased. No plasmoblasts or multinucleated plasma cells were seen. There were many large reticulum cells with phagocytosed material resembling those seen in "metabolic" diseases.

Roentgenologic studies of the skull showed multiple lytic areas within the skull tables; studies of the long bones disclosed extensive destructions involving the lower portions of both femora and the proximal ends of both tibiae. The chest and abdomen appeared normal. An intravenous pyelogram disclosed satisfactory function of both kidneys.

Course in Hospital.—The general debilitated condition of the patient was not in keeping with the severity of the diabetes. A new determination of the BMR could not be performed because of the serious general condition, but there was neither an enlargement of the thyroid nor suggestive eye signs; nor was there hyperirritability, hyperreflexia, or toxic tachycardia. The presence of generalized lymph node enlargement and severe bone pain pointed strongly in the direction of a malignant disease; the latter, particularly to multiple myeloma. The finding of Bence-Jones protein at two urine examinations and the roentgenological evidence of multiple lytic lesions in the skull and the long bones supported this suspicion. The xanthelasmas over the eyelids and the xanthomatous-like eruptions over the trunk were considered as nonrelated phenomena and could be explained as little by the diagnosis of multiple myeloma as could the gingival lesions. Neither was there any hyperglobulinemia nor did the electrophoretic examination of the blood serum reveal the characteristic myeloma pattern. In spite of the absence of diabetes insipidus and exophthalmus, the possibility of Hand-Schüller Christian disease was considered. Yet the final diagnosis was obtained only through bone marrow aspiration and biopsies. Throughout the period of investigation, the diabetes was controlled with diet and insulin; no other therapy was given. When radiotherapy of the bone lesions was considered, the patient developed suddenly, on Jan. 28, 1954, a new episode of severe pain in the spine and the right leg. This time a marked swelling of the femur just above the right knee was noted, and x-ray examination confirmed a pathological fracture of the distal end of the femur. The patient was very weak and appeared moribund. A few days later, a firm mass was noted for the first time in the left hypochondrium, which did not seem to belong to or represent the spleen. The same day the patient became comatose and hemiparetic. There was no acetone in her urine or her breath, the CO_2-combining power was unchanged. Cerebral involvement by the underlying systemic disease appeared most likely. The patient died on Feb. 16, 1954, without regaining consciousness.

Postmortem Examination.—Gross Findings: At postmortem examination the body measured 62 in. (155 cm.) and weighed approximately 75 lb. (34 kg.). There were yellowish-orange plaques in the inner corners of both eyes, involving the upper and lower lids and measuring from 7 to 14 mm. There were also numerous discrete slightly elevated brownish to orange wart-like eruptions on both sides of the neck, chest, and both antecubital fossae, varying from 2 to 4 mm. in diameter. The left upper gingiva disclosed several shallow ulcerations. Numerous discrete and large lymph nodes were palpable in both axillae and groins. The heart weighed 165 gm. It appeared normal except for replacement of the subepicardium by a bright yellow rubbery tissue which caused thickening of the coronary groove up to 8 mm. The aorta disclosed marked extensive atheromatosis of the intima. The adventitia measured up to 8 mm. and was converted into a yellow rubbery tissue. The spleen weighed 360 gm. On section the pulp presented a beefy-red appearance and was completely devoid of the normal architecture. The kidneys each weighed 160 gm. The capsule stripped with ease, revealing finely granular surfaces. Sections through the parenchyma showed a light-brown pale parenchyma. The cortices averaged 4 mm. The pelvic fat of both organs was replaced by a firm bright yellow tissue, which in several areas extended into the cortex (Fig. 1). There was moderate bilateral hydronephrosis. The remaining genitourinary tract was normal except for several large subserous calcified fibroids. Bone: The inner tables of the occipital and both parietal bones disclosed punched-out defects filled by a soft yellow tissue. Sections from the pathologic fracture of the distal end of the right femur revealed extensive replacement of the bone marrow as well as of the cortex by a soft gray tissue mottled with

Fig. 1.—Right kidney. The pelvic fat is replaced by a firm bright yellow tissue which extends into the parenchyma. There is moderate hydronephrosis.

bright yellow areas. The bone marrow of the proximal end of the femur was rubbery and yellow-gray in appearance. The brain weighed 1220 gm. There was a round walnut-sized gray-white rubbery tumor over the right parietal lobe, firmly adherent to the dura and depressing the underlying brain. The enveloping capsule was up to 2 mm. thick and gray-white mottled with bright yellow areas. Cross section through both cerebral hemispheres showed mild bilateral ventricular dilatation. In the region of the right basal ganglia the brain substance was replaced by a yellowish-orange ill-defined moderately firm tissue, measuring approximately 8 mm. in diameter. There was enlargement of the axillary, the inguinal, and the retroperitoneal lymph glands, which measured up to 1.5 cm. in length. The cut surface was gray-white and rubbery. The mesentery contained an ill-defined mass which was located near the root and measured 7 by 4 by 3 cm. On sectioning it consisted of a canary-yellow moderately firm tissue. The lungs, thyroid, liver, pancreas, adrenals, and hypophysis disclosed no gross changes.

Microscopic Findings: Sections through the lymph nodes showed extensive replacement of the parenchyma by proliferating reticulum cells and histiocytes (Fig. 2). There were a few scattered eosinophiles and giant cells noted, but these were scarce and were not a feature. Sudan IV stains failed to disclose evidence of fat. Sections from the spleen revealed almost complete obliteration of the architecture. The normal splenic components were almost entirely replaced by large histiocytes and multinucleated giant cells. Stains with Sudan IV

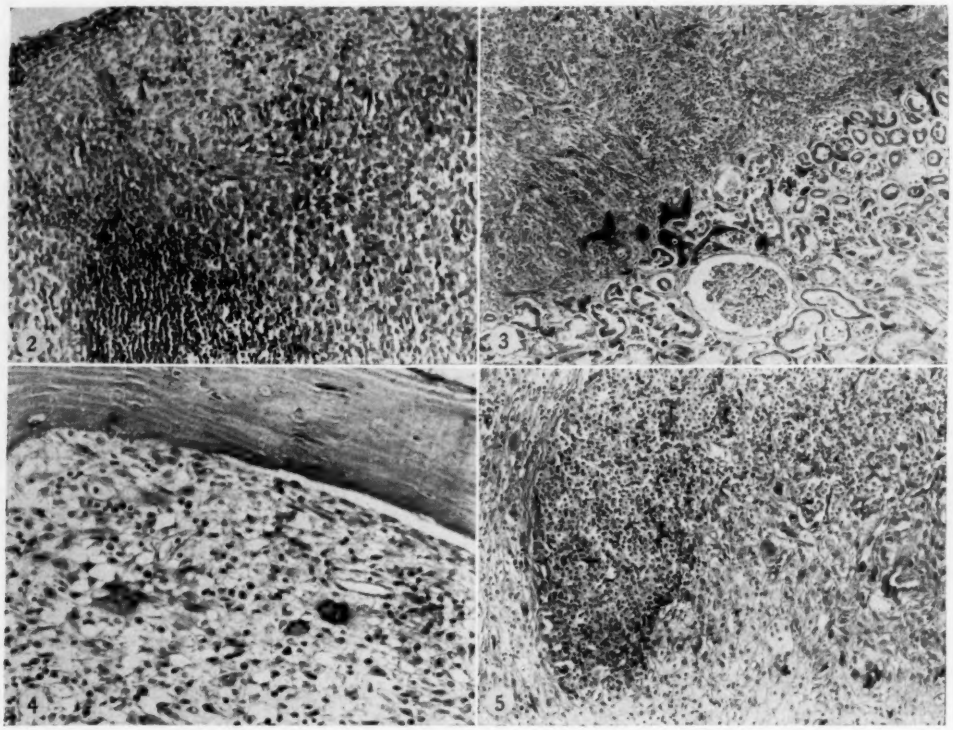

Fig. 2.—Retroperitoneal lymph node. The architecture is obliterated. The parenchyma is extensively replaced by proliferating reticulum cells and histiocytes. Hematoxylin and eosin stain; reduced approximately ⅓ from mag. × 120.

Fig. 3.—Right kidney. A large area of the parenchyma is replaced by a pleomorphic tissue which consists of histiocytes, reticulum cells, fibroblasts, occasional lipophages, and lymphocytes. Hematoxylin and eosin; reduced approximately ⅓ from mag. × 60.

Fig. 4.—Right femur. The bone marrow is replaced by "xanthoma" cells, fibroblasts, occasional multinucleated giant cells, and lymphocytes. Hematoxylin and eosin; reduced approximately ⅓ from mag. × 150.

Fig. 5.—Parathyroid gland. The glandular parenchyma is largely replaced by a granulomatous tissue consisting of "xanthoma" cells, fibroblasts, a few lymphocytes, and occasional giant cells. Hematoxylin and eosin; reduced approximately ⅓ from mag. × 90.

were negative for intraplasmatic fat-staining material. Sections through the kidneys disclosed moderate sclerosis of the small arteries and arterioles. Large areas of the pelvic fat and of the renal parenchyma were replaced by a pleomorphic tissue which consisted of histiocytes, reticulum cells, fibroblasts, and lipophages. There were also few eosinophiles and round cells (Fig. 3). Sudan IV stains disclosed large amounts of intracellular and extracellular fat-staining material, which also gave to a large extent a positive Schultz reaction. There was frequently considerable deposition of collagenous tissue noticeable, as seen in Van Gieson's stains. Sections through the bone (Fig. 4), the brain lesions, the mesenteric mass, the adventitia of the aorta, and the subepicardial tissue revealed granulomatous lesions similar to those described in the kidneys. The lungs, the liver, and one of the parathyroid glands (Fig. 5) contained discrete miliary granulomas consisting of similar cellular components. Sections through the hypophysis, the pancreas, the gastrointestinal tract, the adrenals, and the thyroid disclosed no conspicuous changes.

*Analysis of Lipid Content of Tissue**

	Normal Lymph Node †	Patient B. W.	
		Mesenteric Xanthoma	Periaortic Tissue
Total cholesterol.....	0.9	8.6	10.5
Cholesterol esters.....	0.287	7.4	8.2
Total phospholipids...	4.0	1.9	3.6
Total fatty acids.....	8.0	50.1	33.5
Neutral fats.....	4.2	44.5	26.2

* The values are expressed as percentages of the dry weight of the tissues (Dr. S. J. Thannhauser).
† According to Thannhauser,[29] p. 417.

Lipid Content of Tissue: We are indebted to Dr. S. J. Thannhauser for the chemical analysis of the lipid content of specimens from the mesenteric mass and the periaortic tissue. The data are presented in the Table. Since normal figures for similar kinds of tissue are not available, the data are compared with the lipid content of a normal lymph node as given by Dr. Thannhauser. It is evident that in the patient's tissues there was a marked accumulation of cholesterol, cholesterol esters, total fatty acids, and neutral fats.

Anatomical Diagnosis: 1. Disseminated normocholesteremic xanthomatosis involving both eyelids (xanthelasma), spleen, liver, bones, right basal ganglia, dura mater, aorta, mesentery, subepicardial fat, gingiva, and one of the parathyroid glands. 2. Generalized adenopathy involving the cervical, mediastinal, retroaortic, and mesenteric lymph nodes. 3. Splenomegaly. 4. Pathologic fracture of right femur (distal end). 5. Meningioma. 6. Generalized arteriosclerosis, marked. 7. Coronary sclerosis, marked. 8. Bronchopneumonia of right lower lobe, focal. 9. Pulmonary edema. 10. Multiple fibromyomata of uterus. 11. Cachexia. 12. Diabetes mellitus (clinical). 13. Bilateral hydronephrosis, moderate.

COMMENT

The diagnosis of this case, although suspected clinically, was established by the biopsies and the postmortem examination. While the biopsies disclosed changes consistent with nonlipid histiocytosis or Letterer-Siwe disease, the postmortem examination revealed widespread granulomatous and xanthomatous lesions characteristic for Hand-Schüller-Christian disease. Evidence could be found of almost all features of the different phases which, according to Engelbreth-Holm, Teilum, and Christensen,[28] are characteristic for the gradual evolution of the lesions of eosinophilic xanthomatous granuloma. These authors differentiate between (1) a proliferative phase consisting predominantly of reticulum cells and histiocytes, with accumulation of eosinophiles; (2) a granulomatous phase, with increase of blood vessels, fibroblasts, eosinophilic leucocytes, histiocytes, giant cells (Touton cells), and incipient lipid phagocytosis; (3) a xanthomatous phase, in which aggregations of foam cells containing lipid-staining material became evident, and (4) a fibrous phase, considered a healing stage, in which the granulomatous lesions are gradually replaced by connective tissue. Our findings illustrate particularly well the facts that these four phases do not always show definite demarcation and that their histologic features may overlap within the same lesions or in various stages of the pathologic process.

Although the extent of the lesions in our patient was far greater than is seen usually, it is noteworthy that neither the pituitary, the tuber cinereum, or the orbit were involved. Lesions were present, however, in the cranium and the long bones; in spleen, kidney, and liver; in the aorta, the mesentery, and the mesenteric and retroperitoneal lymph nodes; in the subepicardial fat; in the dura mater and the basal ganglia; in the eyelids, the gingiva, and the skin, and in one of the parathyroid glands.

The clinical manifestations in our patient are at least in part explained by the localization of the lesions. The bone pain and pathological fractures, the abdominal mass, and the late cerebral signs can easily be correlated with the lesions in the involved respective organs. The absence of exophthalmos and of diabetes insipidus can similiarly be explained by the lack of involvement of the orbit, the pituitary, or the tuber cinereum. The question, however, remains undecided whether the diabetes mellitus and the questionable hyperthyroidism were manifestations of the systemic disease or independent complications. No tests for hyperthyroidism were carried out while the patient was under our observations. There was, however, no doubt about her diabetes. Neither pancreas nor thyroid appeared to be involved histologically. The time relationship between the development of glycosuria and hyperglycemia and the first manifestations of the fatal disease is nevertheless suggestive. In at least one other reported case diabetes mellitus was also present.[29] On the other hand, the patient's family history revealed a hereditary predisposition for diabetes.

The widespread distribution of the lesions explains also the differential diagnosis problem. In the absence of the classic triad the diagnosis is always difficult. Even in the relatively rare cases in which skin involvement is present, as in our patient, these skin lesions are not always associated with the underlying process. As in most atypical cases of normocholesteremic xanthomatosis, our differential diagnosis included metastatic carcinoma and multiple myeloma. The roentgenographic studies did not contribute sufficiently to the diagnosis. Although the findings of a uterine mass and the suspicion of an enlarged right kidney suggested a metastatic tumor, this diagnosis was ruled out when these lesions were recognized as fibroids and hydronephrosis. The bone changes as well as the observation of Bence-Jones protein on two occasions seemed to support the suspicion of multiple myeloma, which was dispelled only by the diagnostic lymph node biopsy. This is another illustration of the fact that Bence-Jones proteinuria by itself is not pathognomonic for multiple myeloma. It may occur in other bone diseases, such as metastatic tumor, fibrocystic disease of bone, and osteoporosis as well as in chronic leukemias and in polycythemia.[30] In another recent report of a case of systemic reticulosis, Bence-Jones proteins were also noted to be present in the patient's urine.[31]

Gaucher's, Niemann-Pick, or Hodgkin's disease may sometimes pose differential diagnosis problems in normocholesteremic xanthomatous granuloma as well as isolated bone cysts in the more localized form of the disease. Neither condition needed to be considered in this case.

The coexistence of the acute and chronic lesions of normocholesteremic xanthomatosis, or histiocytosis X, in this aged patient demonstrates again the nosologic entity of this condition. Moreover, it shows that, contrary to the general belief, the acute phase of the disease is not confined to childhood but may occur even in senescence and that the prognosis of the disease depends less on the age at onset than on the character of the process. The acute form seems to have a poor prognosis even in the aged. The incidence of the disease in adulthood and senescence is admittedly rare, but there can be little doubt that in the past a number of cases remained undiagnosed. With better knowledge of the syndrome and the wider use of diagnostic laboratory aides, particularly tissue biopsies, more adult cases come to be recognized. Thannhauser,[29] in 1950, refers to only three adult cases of his own observation; two males and one female, the oldest being 51 years of age. During the past year (1954) alone, four new cases have been reported ‖; all occurred in men with an age range between 30 and 40 years. It seems that the disease has in the adult the same preponderance among the male sex as in children.

The question as to the duration of the disease in our patient may be raised. It is noteworthy that there was a history of progressive downhill course of little more than six months' duration. The lymph node enlarge-

‖ References 26 and 31.

ment and the involvement of the skin and of the long bones developed during this time. On the other hand, the predominantly granulomatous lesions in the viscera as well as the xanthelasma around the eyelids suggest a process of rather long standing. This assumption is supported also by the result of the chemical analysis of some of the mesenteric and periaortic tissues, which disclosed a significant increase of total lipid and fatty acid content. Such xanthomatous transformation may be found, according to Thannhauser, in chronic inflammatory tissue after degenerative processes.

Its occurrence in conjunction with the widespread granulomatous lesions in our patient seems to indicate that it is part of the basic pathologic process, and not simply the result of tissue necrosis. It might therefore be conjectured that the chronic disseminated form of the disease had developed gradually over many years but remained rather asymptomatic until about half a year prior to death, when it suddenly became accelerated and turned into the acute form, involving lymph nodes and the marrow of the long bones.

There is as little known about the cause for this change of the pathological process as about the etiology of the disease itself. It has been mentioned above that the absence of hypercholesteremia sets the disease aside from those lipidoses in which the cholesterol deposition is secondary to a high blood cholesterol level. The serum lipids, especially cholesterol, neutral fats, and phospholipids, are always found within the normal range. This, together with the granulomatous character of the lesions, seems to rule out a disturbance of intermediary fat or cholesterol metabolism and argues in favor of a cellular disorder which manifests itself in an increased formation of cholesterol within the cells, with subsequent intracellular storage of this lipid. The absence of cholesterol and of foam cells in the early or acute lesions of eosinophilic granuloma of bone and of acute histiocytosis implies further that this cellular derangement is not the primary cause of the disease. Thus, Thannhauser [29] proposes that some of the reticulum cells and histiocytes which constitute primarily the proliferative phase of the disease maintain or revert to their embryologic functional possibilities to form lipids and particularly cholesterol. These cells are then tranformed into xanthoma or foam cells.

The predominance of eosinophiles in the acute lesions has given rise to speculations of an allergic etiology. At the same time, a chronic inflammatory process secondary to viral or bacterial infection ¶ or of blastomatous origin [36] has been considered as a possible etiologic factor.

Owing to our lack of knowledge as to the cause of this disease it is not surprising that no effective therapy is available as yet. The localized eosinophilic granuloma of bone is most frequently self-limiting or accessible to surgery. For the disseminated acute or chronic forms various therapeutic procedures have been recommended. Antibiotics have been useful in some instances and disappointing in others #; dietary management with restriction of cholesterol intake seems to be of little value, since the intermediary fat metabolism is not involved. Cortisone has been employed in some cases.[39] The best result seems to be obtained presently with x-ray therapy.* The reticulum cells appear to be most sensitive to radiation. Unfortunately, our patient did not have the benefit of a trial with radiation therapy, since the diagnosis was not made before the terminal state of the disease had been reached.

SUMMARY

A review of the unified concept of the diseases of the reticulum cell system is presented, according to which Hand-Schüller-Christian disease, Letterer-Siwe disease, and eosinophilic granuloma of bone are to be considered as different phases and different manifestations of a single nosologic entity and no longer as independent diseases. The concept is based on the functional interrelationship of the histological lesions in these three conditions. The new entity has been

¶ References 32-35.
# References 31, 37, and 38.
* References 40-42.

called eosinophilic normocholesteremic xanthomatous granuloma (Thannhauser) or histiocytosis X (Lichtenstein).

The clinical course and the postmortem findings of an instance of this disease in an aged patient are reported. This case presents features of at least two of the component conditions and demonstrates the soundness of the new concept.

Dr. Maurice Morrison and the Hematology Service of this hospital cooperated in this study. The photographs were prepared by Mr. Herbert A. Fischler.

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Multiple Myeloma

Review of Sixty-One Proved Cases

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Multiple myeloma is a well-known clinically distinctive malignant disease of the skeleton, characterized by multiple osteolytic plasma-cell tumors of bone and a proliferation of plasma cells within the marrow. These tumors have a predilection for the bones where active blood formation takes place in the adult; i. e., the sternum, ribs, vertebral bodies, skull, bones of the shoulder girdle, pelvis, and upper ends of the humeri and femora.

Although the substance now known as Bence-Jones protein was first discovered in 1845 by Dr. Henry Bence-Jones [1] and von Rustizky [2] first described the condition *multiples Myelom* in 1873, it was not till 1889 that Kahler [3] associated this disease with the occurrence of Bence-Jones proteinuria. Since then, excellent reviews by Geshickter and Copeland,[4] Bayrd and Heck,[5] and Lichtenstein and Jaffe [6] have helped systematize our present knowledge of this disease.

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This discussion is based on 61 cases seen at the Winnipeg General Hospital, Deer Lodge Hospital, and the Municipal Hospitals from 1946 to 1954. This series includes 21 cases from the Winnipeg General Hospital which have been reported previously.[7] All cases have been proved by biopsy, marrow aspiration, or at necropsy.

These 61 cases have been arbitrarily divided into two groups on clinical grounds: (1) cases with typical symptomatology and findings, 48 in number, and (2) cases with somewhat atypical symptomatology and findings, 5 in number: (*a*) 3 presented with a refractory anemia, (*b*) 1 presented with macroglossia, and (*c*) 1 presented with a painless atrophy.

The remaining eight cases consist of one plasma-cell leukemia and seven solitary myelomas and are included in the total series because multiple myeloma, solitary myeloma, and plasma-cell leukemia are considered as variants of the same disease.

The cases listed as solitary myeloma are so labeled because of their presentation. In one case the bone

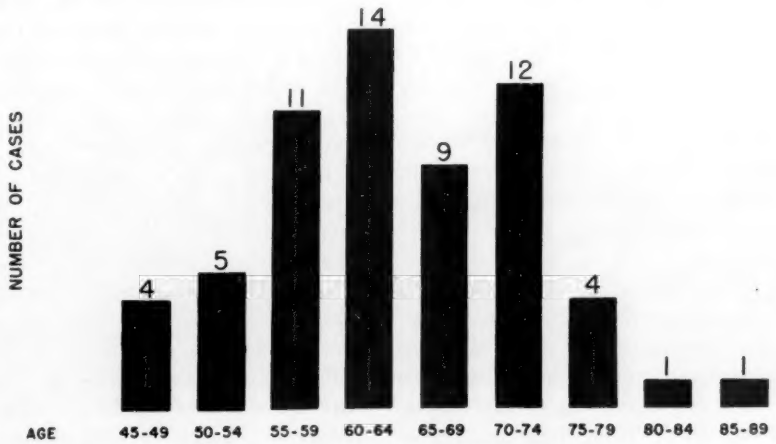

Fig. 1.—Age incidence in multiple myeloma.

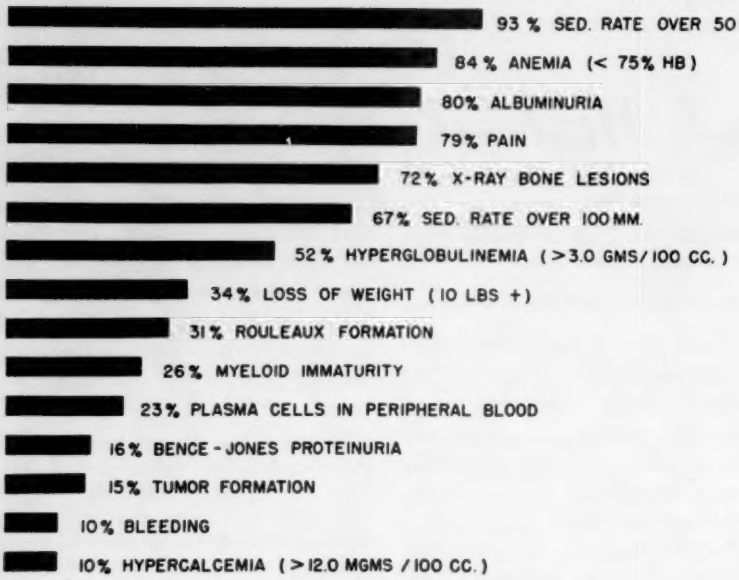

Fig. 2.—Symptoms in multiple myeloma.

lesions became multiple before death. In four cases the sedimentation rate was over 50 mm. Hyperglobulinemia was present in two of these, and albuminuria, in three. In the sixth case there was marked anemia and hyperglobulinemia. In the seventh case autopsy revealed a myeloma kidney and the ilium also showed myeloma, but sternal and vertebral marrows were not checked. There is, therefore, in all our cases of solitary myeloma, some indirect evidence that the disease was diffuse.

Incidence: The age incidence is shown in Figure 1. One half of our cases fell between the ages of 50 and 64. Seventy per cent were males, the exact incidence being 43 males and 18 females. This sex incidence is prejudiced somewhat by the fact that 14 of our cases came from the D. V. A. hospital.

CLINICAL FINDINGS

The two commonest entrance complaints were pain and loss of weight. Other symptoms, including bleeding, tumor formation, large tongue, and arthritis, were relatively infrequent.

Pain: Forty-eight of our cases, or 79% of the series, presented initially the complaint of pain. In the majority of these, the pain was persistent, progressive, and situated in the low back region. It was usually aggravated by movement, lifting, coughing, sneezing, and straining. In three cases the pain was sciatic in nature, and in one other case it was an upper dorsal root pain.

A loss of weight of more than 10 lb. (4.5 kg.) was noted in 21 cases, or 34%. In some cases this weight loss was extreme.

Bleeding was observed in six cases, or 10%. In four of these patients this took the form of epistaxis, and in two others, of rectal bleeding. In two of these patients, who had platelet counts done, the figure was below 40,000. In five of the six cases hyperglobulinemia was noted, and in the sixth case the proteins were not checked.

Tumor formation was recorded in nine cases, or 15%. Six of these cases are among those with the solitary myelomas.

Macroglossia, found in two cases, was considered to be due to amyloidosis, but, unfortunately, in neither case is biopsy or autopsy proof available. Amyloid deposits in association with multiple myeloma often occur in unusual sites such as bone, muscle, connective tissue, skin, tongue, gastrointestinal tract, etc. Lichtenstein and Jaffe,[6] finding atypical amyloidosis in 10% of 35 cases of multiple myeloma, suggest that whenever primary or atypical amyloidosis is found the possibility of myeloma should

be investigated. In 24 of our cases that underwent autopsy, atypical amyloidosis was noted in 3, or 12%, and typical or secondary amyloidosis was noted in 2, or 8%.

Neurological manifestations were exhibited in seven cases, or 11%, of the series. Besides the three cases of sciatica and the case of upper dorsal root pain previously mentioned, one patient presented with signs of extramedullary spinal cord compression and two presented with the picture of a peripheral neuritis. Neural complications have been noted in as high as 40% of cases in certain reported series [8] and consist of intracranial lesions, lesions of the cord, lesions of the nerve roots, compression of the spinal ganglia, and peripheral neuritis.

The blood picture in this series of cases revealed certain significant changes, including (1) elevation of the sedimentation rate, (2) anemia, (3) rouleaux formation, (4) plasma cells in the peripheral blood, and (5) myeloid immaturity.

1. Elevation of the sedimentation rate over 50 mm. (Westergren) was noted in 57 of our cases, or 93%. The sedimentation rate was over 100 mm. in 41 cases, or 67%. In two cases the sedimentation rate was abnormal but under 50 mm., and in two cases it was not checked. In 28 of the 41 cases with sedimentation rates over 100 mm. there was associated hyperglobulinemia.

2. Anemia of the order of 11.9 gm. hemoglobin (75%) or less was recorded in 51 cases, or 84%. Three cases presented with a macrocytic anemia superficially resembling pernicious anemia but refractory to liver or vitamin B_{12} therapy. In the majority of cases, however, the anemia was normocytic or slightly microcytic. The anemia is said to be due mainly to progressive neoplastic encroachment on the marrow, but it may also be related to bleeding into tumor tissue, toxic inhibition, the effects of general debilitation, and renal damage. It is notably refractory to any form of therapy.

3. Rouleaux formation, the presence of plasma cells in the peripheral blood stream, and myeloid immaturity, as far back as the stem cell, were all observed with less frequency but sufficiently often to warrant mention. Rouleaux formation occurred in 19 cases (31%); plasma cells, in 14 cases (23%), and myeloid immaturity, in 16 cases (26%). Rouleaux formation bears a close relationship to elevated sedimentation rates and hyperglobulinemia. Autohemagglutination in Haymen's solution is also frequently observed in association with rouleaux formation.

The two significant biochemical changes in multiple myeloma are hyperglobulinemia and hypercalcemia.

Hyperglobulinemia of 3 gm/100 cc. or more was noted in 32 cases, or 52%, the highest globulin recorded being 13.6 gm. In the past 10 years, electrophoresis and chemical methods of fractionation of proteins have been utilized. In this series 14 cases have had fractionation of serum proteins by a combination of salting out and turbidity determinations.[9] Of these 14 cases, protein fractionation showed an increase in gamma globulin in 9 cases, an increase in beta globulin in 2 cases, and an increase in alpha globulin in 1 case. In two cases serum protein fractionation was normal. Cryoglobulinemia was encountered in only one case and was associated with a very high gamma globulin level. Bence-Jones proteins have been chiefly associated with the beta globulin fraction [10] or with the fractions moving with an electrophoretic velocity between that of the beta and gamma globulins.[11]

Although it is now generally accepted that the plasma cells in myeloma are the cause of the hyperglobulinemia and probably also of the cryoglobulinemia, if present, it must be remembered that these abnormalities can occur in other varied disease states. Hyperglobulinemia has been noted in cases of kala-azar, sarcoidosis, lymphogranuloma venereum, and severe liver damage. Cryoglobulins * have been noted in lymphatic leukemia, subacute bacterial endocarditis, bronchiectasis, disseminated lupus, and rheumatic fever, as well as the above.

* References 12-15.

Hypercalcemia, with values for serum calcium of more than 12.0 mg/100 cc., was found in six cases, or 10%. However, in one-third of the series calcium estimations were not done. This increase in calcium is due to the lytic resorption of bone and is also related to the tendency toward renal failure in many cases.

In determining whether myeloma or hyperparathyroidism is the cause of hypercalcemia in any given case, it should be noted that the serum alkaline phosphatase activity tends to be normal in myeloma and increased in hyperparathyroidism.[16] In this series the alkaline phosphatase was checked in 16 cases and found to be normal in 15.

The chief renal complications of myeloma are albuminuria and Bence-Jones proteinuria.

Albuminuria was noted in 49 cases, or 80%. The blood urea nitrogen was found to be elevated in 16 cases, or 26%. At autopsy, the kidneys characteristically show tubular degeneration, with plugging of the tubules by dense eosinophilic hyaline-like plugs of proteinaceous material. These changes are thought by some [17] to be part of the terminal picture of renal failure, while the standard texts [18] still consider the casts to be the cause of a degenerative atrophy of the tubules and subsequent renal failure. Whatever the cause of the renal failure, it is noteworthy that hypertension and edema are rarely associated with this type of renal insufficiency.[18] In none of our patients with elevated blood urea nitrogen was either hypertension or edema noted. The highest recorded diastolic pressure in this group was 90 mm., and the highest systolic, 160 mm.

Bence-Jones protein was found in the urine of only 10 of our cases (16%). This is in marked contrast to most series, which report its incidence as around 50%.† As Bence-Jones proteinuria is transient in nature, and as three or fewer examinations were made on the urine in the majority of our cases, this is the probable explanation of our low incidence.

† References 4-6.

Bence-Jones protein is precipitated from urine by heating it to temperatures between 40 and 60 C. When the temperature is raised still higher, it redissolves, to appear again on cooling. Recently a simple ring test [19] and a precipitin test [20] have been developed in an attempt to pick up Bence-Jones protein in even minute quantities. This abnormal protein has a mean molecular weight of 37,000 (showing variations from 24,000 to 90,000). It probably represents an alteration in the abnormal serum proteins seen in multiple myeloma and presumably originates in the plasma cells.[21] Bence-Jones protein is found but rarely in other conditions such as metastatic carcinoma and sarcoma of bone, lymphatic and myelogenous leukemia, and polycythemia.

X-ray changes in the skeleton are, if multiple, indicative of myeloma. Roentgenograms were abnormal in 44 of our cases (72%). In 16 cases the typical punched-out discrete osteolytic lesions were noted, while in 20 cases the changes were chiefly those of osteoporosis. Eight cases showed solitary destructive lesions.

CAUSATION AND CYTOLOGY

The various aspects of myeloma give this disease a resemblance to forms of cancer, leukemia, or lymphoma. Waldenström [22] feels that present evidence is in favour of myeloma being a systemic disease allied to leukemia rather than a classic malignant neoplasia. Most workers now agree with the concept of Diggs and Sirridge [23] that this disease is represented by a single cell type arising from reticulum cells in a dysplastic plasma cell line. It is generally accepted that the type cell in myeloma is an immature plasma cell and that other types of myeloma described in the literature probably represent plasma cell myelomas with a leukemoid reaction. All of our cases were plasma cell myelomas. Marrow examination was diagnostic in 49 out of 50 cases. Diagnosis was made by biopsy in eight cases and at autopsy in four others.

DIAGNOSIS

The presence of abnormal plasma cells or myeloma cells on marrow aspiration or biopsy constitutes the only absolute criterion of multiple myeloma. The diagnosis should be considered if any of the following findings are noted: musculoskeletal pain, especially if associated with a marked weight loss; obscure renal disease without hypertension; refractory anemia without obvious etiology; severe osteoporosis or more classic x-ray findings such as discrete osteolytic lesions; Bence-Jones proteinuria; hyperglobulinemia due to increase of any of the globulin fractions; hypercalcemia; rouleaux formation; high sedimentation rate, and the finding of unusual tumor-like amyloid deposits.

Marrow aspiration should be employed freely in obscure diagnostic problems with any combination of the above, but one must remember that the changes in the marrow may be irregularly distributed and that, therefore, a negative result on marrow examination does not exclude myeloma.

In the differential diagnosis with multiple myeloma both metastatic bone lesions and hyperparathyroidism have to be considered. These are usually easily differentiated by the difference in chemical changes and by marrow aspiration. Waldenström's macroglobulinemia ‡ also enters into the differential diagnosis. This is a relatively benign condition usually affecting elderly men. The main complaint is not bone pain but rather lassitude, dyspnea, and bleeding from mucous membranes. These patients often exhibit pallor and scattered nontender enlarged lymph nodes. X-rays never show multiple punched-out lesions but occasionally reveal general demineralization. These patients have anemia and an extremely high sedimentation rate. Marrow aspiration does not reveal abnormal plasma cells but rather large numbers of small lymphocytoid cells with shedding of protoplasm. The final diagnosis rests with the ultracentrifuge and the demonstration of the presence of serum globulins having an abnormally high molecular weight.

‡ References 22 and 24.

TREATMENT

There is nothing of proved curative worth to be offered to patients with myeloma. Palliative x-ray treatment, blood transfusions, and urethan are the mainstays of therapy at present. Stilbamidine is rarely if ever now used, and corticotropin and cortisone have not proved themselves of any other than temporary symptomatic value.

Urethan therapy is of some value in symptomatic treatment. Luttgens and Bayrd [25] report on its use in 53 cases over a two-and-a-half-year period. Fifty per cent of their patients felt symptomatically better, but only 20% were objectively improved. There is sometimes a slight increase in hemoglobin and/or a decrease in sedimentation rate. No great change in serum proteins, x-ray findings, or the percentage of myeloma cells in the marrow occurred. Our own results with urethan have not been so gratifying.

While these results are not too encouraging, administration of urethan is probably as good treatment as is available for myeloma at the present time.

PROGNOSIS

Of the 61 patients in this series, 54 have died, 5 are still alive, and no follow-up was obtained on 2. Twenty-nine patients died within one year of the onset of their symptoms, and a further 16 died within two years. The average interval between onset of symptoms and death in the 54 cases was just over one year. Those cases presenting with solitary lesions and those presenting with refractory anemia had the longest survival times: four years and four months, and five years and six months, on an average, respectively.

SUMMARY

Sixty-one cases of pathologically proved multiple myeloma have been reviewed, with a discussion of clinical and laboratory data.

Emphasis has been placed on marrow aspiration as the most reliable confirmation of a diagnosis of multiple myeloma.

Dr. John Judge gave assistance in the preparation of this paper.

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Plasma Thromboplastin Antecedent Deficiency

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Until recently, male persons with a severe hemorrhagic diathesis characterized by (1) a prolonged clotting time, (2) normal Quick plasma prothrombin time, but (3) a poor prothrombin consumption were unhesitatingly diagnosed as suffering from classic hemophilia. This disease is still widely attributed to a deficiency of antihemophilic factor (AHF)[1] essential for the formation of blood thromboplastin. However, it has been emphasized that the demonstrable decrease of AHF activity in these patients may be functional rather than real, owing to the presence of an inhibitor (antithromboplastin).[2]

In 1952, Aggeler and co-workers[3] described a syndrome, clinically and genetically indistinguishable from classic hemophilia, which is apparently caused by the absence or marked diminution of a previously unrecognized plasma constituent. They named this factor plasma thromboplastin component (PTC). PTC is also essential for the formation of blood thromboplastin. The PTC-deficiency syndrome is now known under a variety of names, such as hemophilia B,* Christmas disease,[6] or deuterohemophilia.[7]

Since PTC is adsorbed by $BaSO_4$ from oxalated plasma,[8] in contrast to AHF, and since aged serum contains PTC but not AHF,[8] a simple way presents itself to distinguish these two disorders. Addition of these reagents to the patient's blood selectively corrects the abnormal clotting and serum prothrombin times. About 20% of all patients previously diagnosed as having classic hemophilia have actually a PTC-deficiency syndrome.†

In 1953, Rosenthal and co-workers[11] drew attention to another hemorrhagic diathesis, resembling hemophilia, which could not be classified as either AHF or PTC deficiency, since the prolonged clotting and serum prothrombin times of these bloods were corrected by both, $BaSO_4$ plasma as well as serum. These investigators postulated a deficiency of a third thromboplastic factor, named plasma thromboplastin antecedent (PTA), in these patients. PTA is also necessary for the generation of blood thromboplastin, as are AHF and PTC. The clinical manifestations of the PTA-deficiency syndrome were stated to be mild and to occur in either sex.‡

This paper reports three instances of PTA deficiency. Two patients were women whose hemorrhagic diathesis had defied precise characterization in the past. The third is a man in whom the existence of a PTA deficiency could only be demonstrated by means of the sensitive thromboplastin-generation test.[12] Aside from the clinical studies, investigations of the physicochemical properties of the PTA factor were also performed.

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* References 4 and 5.
† References 9 and 10.
‡ References 9 and 11.

METHODS

Of the methods used in this study, two may require some special comment.

1. The one-stage serum prothrombin time,[12] using serum obtained 60 minutes after the clotting of the blood sample kept at 37 C, provides an indication of the transformation of prothrombin to thrombin. Values below 20 seconds reveal a poor prothrombin consumption.

2. The thromboplastin-generation test[13] is a procedure in which the rate and amount of blood thromboplastin formed from certain basic constituents is measured. A mixture of platelets, oxalated plasma adsorbed by $BaSO_4$, and serum are used and added to a substrate. The substrate consists of citrated platelet-poor plasma. After recalcification the clotting time of the substrates is determined. In this manner the generation of thromboplastin can be followed. Should the development of thromboplastin be insufficient, substitution experiments are performed, replacing the various components of the mixture with corresponding normal preparations.

REPORT OF CASES

PATIENT 1.—Mrs. S. A., aged 49, was considered to be a poor surgical risk, since she had experienced profuse hemorrhages after any dental or surgical procedure. However, she had never noted any "spontaneous" bleeding. In 1928, following the birth of her only child, she had a severe postpartum hemorrhage. Ten years later, she had a miscarriage. After the gynecologic surgery she continued to show vaginal bleeding for about nine months. In 1939, a tonsillectomy was performed and several blood transfusions were required before an alarming postoperative hemorrhage was brought under control. Several years later, after a tooth extraction, she bled so profusely that she again needed several transfusions. Recently, a nodule in one of her breasts was noted, necessitating biopsy. Because of her history, she was referred to this department for diagnosis of her hemorrhagic diathesis. Previous hematologic examinations at other hospitals had been noncontributory, except that on several occasions a moderately prolonged clotting time had been found. No other members of her family had, or have shown a similar tendency to hemorrhage.

Physical examination did not reveal any abnormality except for the mammary lesion. Laboratory findings: Urinalysis, negative. Blood: Hb., 14.4 gm. per 100 cc.; RBC, 4,600,000; WBC, 5600; hematocrit level (Wintrobe), 42%. Normal differential count. Platelet level, 300,000 (Dameshek's method[14]). Bleeding time (Duke), 4 minutes. The results of the initial clotting studies are compiled in Table 1. As can be seen, the clotting time was slightly prolonged; the plasma prothrombin time was normal, but the serum prothrombin time was definitely abnormal. Since the presence of a circulating anticoagulant could be ruled out,[15] these findings suggested a disturbance of her blood thromboplastin formation, which could be demonstrated objectively by the thromboplastin-generation test (Fig.). Special substitution studies with this procedure permitted the diagnosis of PTA deficiency.

PATIENT 2.—Mrs. I. S., aged 48, shows a history quite similar to that of the first patient. She never experienced spontaneous hemorrhages but bled profusely after tooth extractions, deliveries, and surgical procedures. In 1943, she underwent a cholecystectomy, which was apparently followed by internal hemorrhages, and she received as many as 20 transfusions. In 1947, a hysterectomy was performed, and several transfusions had to be given postoperatively. Severe bleeding also complicated the repair of a rectovaginal fistula in 1948. At that time it was noted that single transfusions stopped the hemorrhage for a few days, but then bleeding reappeared, requiring another transfusion, and this cycle repeated itself for almost two months. Recently, she again had some minor vaginal bleeding and was brought to this department for evaluation of her hemorrhagic diathesis. The patient stated that numerous hematologic examinations in the past had only shown a slight prolongation of her clotting time. Her mother was known to be a "bleeder." Two of her brothers are also considered to have a hemorrhagic tendency. Studies of these two persons demonstrated that one has a mild PTA deficiency (Patient 3) and the other is normal. None of the patient's four children exhibits any bleeding tendency.

TABLE 1.—*Routine Clotting Studies in Three Patients with PTA Deficiency*

Type of Test	Case 1 S. A.	Case 2 I. S.	Case 3 H. K.
Clotting time (Lee White): normal, up to 20 minutes	23-31	19-42	14-18
One-stage prothrombin time: normal, 11 to 12 seconds	12	11.4	11.5
One-stage serum prothrombin time: normal, more than 20 seconds	11.4	11.5	27.0
Circulating anticoagulant in glass		Negative	

Physical examination was essentially negative. Laboratory studies: Urinalysis, negative. Blood: Hb., 13.6 gm. per 100 cc.; RBC, 4,300,000; WBC, 5600; hematocrit level (Wintrobe), 41%. Differential count, within normal ranges. Platelet count, 393,000. Bleeding time, 3 minutes. The results of the clotting tests are summarized in Table 1. The clotting time was first elevated to 29 minutes, but on later occasions it varied from 19 to 42 minutes.

The Quick plasma prothrombin test was normal. The one-stage serum prothrombin time was 11.5 seconds, indicating a poor prothrombin consumption. There was no evidence for a circulating anticoagulant. The thromboplastin-generation test revealed the expected disturbance in the blood thromboplastin formation, and special substitution experiments documented the presence of PTA deficiency.

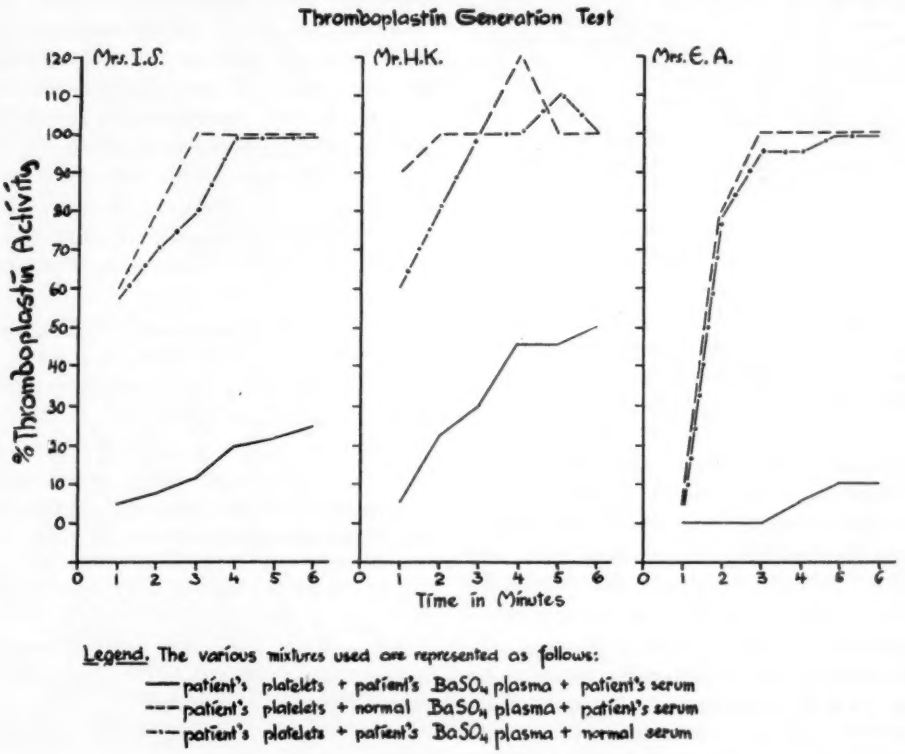
PATIENT 3.—Mr. H. K., aged 54, the brother of Patient 2, also had experienced severe hemorrhages after tooth extractions. He had not undergone any surgical procedure. However, bleeding ulcer was diagnosed several years ago and was treated successfully by a single blood transfusion in addition to the established management for this condition.

Physical examination was negative, as were the laboratory tests. The results of the routine clotting studies are presented in Table 1. As can be seen, no abnormality was demonstrable. However, the thromboplastin-generation test showed a definite decrease in thromboplastin formation (Fig.), and pertinent substitution experiments revealed the existence of PTA deficiency.

SPECIAL INVESTIGATIONS

1. *Effect of Normal $BaSO_4$ Plasma and of Serum on Clotting and Serum Prothrombin Times of the Patients' Bloods.*—These addition experiments were performed only with the bloods of Patients 1 and 2, which exhibited abnormal values in these tests. As can be seen from Table 2, both reagents normalized the results. These findings establish that these patients have a deficiency neither of AHF nor or PTC, since no selective correction by only one of these reagents was found.

2. *Effect of $BaSO_4$ Plasma from a Hemophilic Patient and of Serum from a Patient with Known PTC Deficiency on Clotting and Serum Prothrombin Times.*—In severe hemophilia, oxalated plasma does not contain AHF at all, or only in insignificant amounts. Since $BaSO_4$ adsorbs PTC, the



Thromboplastin-generation test.

TABLE 2.—*Effect of Normal and Hemophilia $BaSO_4$ Plasma and of Normal and PTC-Deficiency Serum on Clotting and Serum Prothrombin Times of Our Patients*

Type of Test	Case 1 S. A.	Case 2 I. S.
Clotting time, minutes		
Without any addition	31	45
Addition* of saline	30	41
Addition of normal $BaSO_4$ plasma	8	6½
Addition of hemophilic $BaSO_4$ plasma	9	7
Addition of normal aged serum	6½	6½
Addition of PTC-deficiency serum	6½	8
Serum prothrombin time, seconds		
Without any addition	11.4	11.5
Addition of saline	11	12
Addition of normal $BaSO_4$ plasma	44	60
Addition of hemophilic $BaSO_4$ plasma	44	40
Addition of normal aged serum	39	39
Addition of PTC-deficiency serum	45	56

* All addition experiments were performed by adding 0.1 cc. of the reagent to 0.9 cc. of the tested blood.

$BaSO_4$ plasma from a person with severe hemophilia does not contain either AHF or PTC. This plasma is, therefore, incapable of correcting in vitro the prolonged clotting and serum prothrombin times of bloods obtained from patients with either AHF or PTC deficiency. Similarly, the aged serum of a person with severe PTC deficiency does not contain this factor, although it is demonstrable in the serum of a normal person as well as in that of hemophilic persons. Pertinent addition experiments with these special reagents, obtained from well-studied persons with these syndromes, demonstrated complete correction of the prolonged clotting and serum prothrombin times of the bloods of Patients 1 and 2 (Table 2). These findings indicate the presence of a factor different from AHF and PTC, which was lacking in the blood of these patients.

3. *Effect of the Patients' $BaSO_4$ Plasma and Serum on Clotting and Serum Prothrombin Times of Persons with Severe Hemophilia and PTC Deficiency.*—As might be expected, the $BaSO_4$ plasma of our patients rectified the clotting abnormalities in well-established cases of severe hemophilia and their sera were effective in persons with severe PTC deficiency. These findings prove that the bloods of our patients were lacking neither in AHF nor in PTC.

4. *Results of the Thromboplastin-Generation Test* (Fig.).—Biggs and co-workers§ have demonstrated that for the formation of complete blood thromboplastin the interactions of PTC, AHF, and platelets, as well as labile (accelerator globulin, proaccelerin, Factor V) and stable (proconvertin, SPCA, Factor VII) factors, are required. The previously described experiments have shown that the plasma of our patients contains PTC and AHF. From their normal plasma prothrombin time it may be deduced that sufficient amounts of labile and stable factors are always also present. Nevertheless, the results of the thromboplastin-generation tests were definitely abnormal (Fig.). In Patient 3 this test was the only one revealing a decreased thromboplastic activity, about 40% of the normal value. Substitution of the patients' platelets by normal platelets did not influence the results. However, substitution of either the patients' $BaSO_4$ plasma or serum by a similar normal preparation rectified the thromboplastin generation. Thus, these findings again indicate that the missing factor, PTA, is normally present in both, $BaSO_4$ plasma as well as serum, and is indispensible for the formation of effective blood thromboplastin.

§ References 13 and 16.

TABLE 3.—*Effect of Patients' $BaSO_4$ Plasma and Serum on Clotting and Serum Prothrombin Times of Persons with Severe Hemophilia and PTC Deficiency*

Type of Test	Hemophilia	PTC Deficiency
Clotting time, minutes	240	120
After addition* of $BaSO_4$ plasma		
Patient 1	9	120
Patient 2	11	120
After addition of serum		
Patient 1	180	12
Patient 2	180	10
Serum prothrombin time in seconds	12.4	13.4
After addition of $BaSO_4$ plasma		
Patient 1	29	13
Patient 2	42	14
After addition of serum		
Patient 1	12	31
Patient 2	12.8	39

* All addition experiments were performed by adding 0.1 cc. of reagent to 0.9 cc. of the tested blood.

PHYSICOCHEMICAL PROPERTIES OF PTA FACTOR

We have determined some of the physicochemical properties of the PTA factor in the course of our investigations, using the serum prothrombin time of the patients' bloods as an indicator of the activity of this factor. During the preparation of this communication, Rosenthal[17] has published similar studies. Table 4 compares his findings with ours. As can be seen, in general there is good agreement of the results of both laboratories. We have, however, not observed an increase of the PTA activity in cold-stored normal plasma. Furthermore, the 0% to 20% ammonium sulfate fraction exhibited in our hands marked PTA activity (Table 5), whereas Rosenthal found only minimal activity. Whether or not these discrepancies are due to the use of different techniques is not clear.

In our addition experiments, 0.1 cc. of the reagents was added to 0.9 cc. of the patient's blood, and the change in the serum prothrombin time was observed. The fractions were prepared by adding saturated ammonium sulfate solutions, adjusted to pH 7.5, to the plasma at 4 C to obtain the final concentration desired. The precipitated plasma was kept for one hour in the refrigerator. The sample was then spun in the refrigerated centrifuge at 3000 rpm for 20 minutes. After washing the precipitates with ammonium sulfate of the same concentration, the fraction was reconstituted with saline solution to the original volume of plasma and then dialyzed at 4 C against large amounts of saline solution until the test for SO_4 ions became negative. This was usually obtained after about five hours. The precipitate was then either tested immediately or kept frozen at —20 C until used.

TABLE 5.—*Influence of Various Plasma Fractions Obtained with Ammonium Sulfate on Serum Prothrombin Time of Patient 2*

Preparation	Serum Prothrombin Time, Seconds
Patient's blood	14.2
Patient's blood + saline	14
Patient's blood + 20% $(NH_4)_2SO_4$ fraction	
Fresh	40.4
After 5 weeks' storage	29.6
Patient's blood + 20%-33% $(NH_4)_2SO_4$ fraction	
Fresh	60
After 5 weeks' storage	33
Patient's blood + 33%-50% $(NH_4)_2SO_4$ fraction	
Fresh	22.8

COMMENT

The three patients described in this communication exhibit a syndrome characterized clinically by the absence of spontaneous bleeding but occurrence of hemorrhages following surgical procedures. Two of them show prolonged clotting time and normal plasma but abnormal serum prothrombin time. In all three, the thromboplastin-generation test reveals a marked decrease in the formation of blood thromboplastin.

TABLE 4.—*Physicochemical Properties of PTA*

Observation	Rosenthal's Findings	Our Findings
Influence of storage at —20 C on PTA activity of		
Normal plasma	Increased	No change
Moderate PTA-deficiency plasma	Increased	Not performed
Plasma fractions prepared by variable saturations with $(NH_4)_2SO_4$	Not performed	Slightly decreased after 5 weeks' storage
Effect of temperature on PTA activity of normal plasma or serum	60 C for 20 minutes; definitely decreased	58 C for 10 minutes; markedly decreased
PTA activity of plasma following absorption with $BaSO_4$ and elution with 3.2% citrate	Present in eluate	Present in eluate
PTA activity after Seitz filtration	Slightly to moderately decreased	Markedly decreased
PTA activity of various $(NH_4)_2SO_4$ fractions	Saturation 0%-25% minimal 25%-33% maximal 33%-50% small amounts	Saturation 0%-20% marked 20%-33% maximal 33%-50% small amounts
Presence of PTA in Cohn's plasma fractions	I and III, small amounts IV_1, maximal II, IV_4, V, none	I, variable*

* One preparation of Fraction I, obtained through the courtesy of the late Dr. E. Cohn, of the Harvard Physico-Chemical Institute, was very active. An experimental sample obtained from the Michigan Department of Health was inactive; Dr. H. D. Anderson supplied this sample.

The pattern of prolonged clotting time and normal plasma but abnormal serum prothrombin times is encountered in all fully developed conditions which have as a common denominator the inability to produce sufficient blood thromboplastin. This pattern has been found in the following entities: (1) classic hemophilia (hemophilia A) due to the nonavailability of AHF; (2) PTC deficiency (Christmas disease, hemophilia B, deuterohemophilia); (3) PTA deficiency; (4) presence of a circulating anticoagulant (hemophilia-like disease), and (5) a disorder, described by Spaet and co-workers [18] in one patient, which may be due to the absence of still another plasma constituent, possibly also necessary for blood thromboplastin formation.

Of these five disorders, three are at present known to be genetically conditioned. AHF and PTC deficiency are due to a sex-linked characteristic and are found in the heterozygous male and, in extremely rare instances, in homozygous females.|| According to Rosenthal,¶ and confirmed by Frick's [21] and our own observations, PTA deficiency occurs in either sex. From that it follows that, after a circulating anticoagulant has been excluded, PTA deficiency may be strongly suspected in a female with a hemorrhagic diathesis showing the above-mentioned laboratory features. Objectively, differentiation of the three genetic disorders can be made by simple addition tests. Normal plasma, adsorbed with $BaSO_4$, contains AHF and PTA. Normal aged serum contains PTC and PTA. Selective correction of the abnormal clotting and serum prothrombin times by either $BaSO_4$ plasma or by serum is diagnostic for hemophilia or PTC deficiency, respectively. Correction by either reagent is diagnostic for the PTA syndrome. However, in mild deficiencies the more sensitive thromboplastin-generation test may be the only procedure to detect an existing anomaly of thromboplastin formation. This has been observed in mild PTC deficiency # and is documented for PTA deficiency by the third case reported in this paper.

The thromboplastin-generation test may also be of value for the differentiation of a PTA deficiency from a combined AHF and PTC deficiency. If this latter syndrome occurs, it is extremely rare.[5] Since AHF, PTC, and PTA are essential for the formation of a complete blood thromboplastin, it can be predicted that in combined AHF plus PTC deficiency, substitution of either the patient's $BaSO_4$ plasma or serum by a normal homologous preparation will not rectify the result of the thromboplastin-generation test. Normalization will, however, be found in PTA deficiency.

Distinction between PTA deficiency and the syndrome described by Spaet and co-workers,[18] attributed to the lack of a possible fourth thromboplastic factor, may also be made by appropriate addition experiments. Spaet and co-workers [18] demonstrated that mixing the blood of their patient with that of a person with established PTA deficiency produced mutual corrections of the prolonged clotting and serum prothrombin times.

In studying some patients with PTA deficiency we have seen marked fluctuations in their clotting times but not in their serum prothrombin times. A similar behavior of the results of these tests can be observed in persons with AHF or PTC deficiency. It is interesting to note, however, that the disease may express itself, clinically as well as in the laboratory findings, in a variable degree of severity in various members of the same family. This is clearly discernible in our Cases 2 and 3. In classic hemophilia and in PTC deficiency, a variable degree of severity of the syndromes also exists, but the degree of severity is remarkably constant in members of the same sibship.* More extensive observations are necessary to find out whether this is a differentiating feature of the PTA syndrome.

|| References 19 and 20.

¶ References 9 and 11.

References 22 and 23.

* References 1, 24, and 25.

Rosenthal has emphasized in his preliminary reports on PTA deficiency † that transfusions exert a more prolonged corrective effect in this disorder than is seen in classic hemophilia. Some observations mentioned in the history of Case 2 seem to corroborate this statement.

The exact role which PTA plays in the gene action of blood thromboplastin is not clear at present. It is difficult to isolate PTA completely from other factors essential for thromboplastin formation, such as AHF, PTC, and the labile and stable factors also involved in the alteration of prothrombin to thrombin. However, the fact that maximal PTA activity is found in the 20% to 33% ammonium sulfate fraction of plasma, whereas the corresponding fractions for AHF and PTC are the 0% to 20% ‡ and the 45% to 50%,[8] respectively, indicate that PTA is a definite clotting factor in its own right. Further studies directed towards obtaining highly purified PTA fraction are needed to elucidate the exact interrelationship of PTA to the other factors necessary for maximal thromboplastin generation.

Meanwhile, physicians must become alerted to the existence of this special type of hemorrhagic disorder. The recognition, especially of the milder forms of PTA deficiency, is of practical significance for the adequate preparation of such patients for surgical interventions.

SUMMARY

Three patients exhibiting the syndrome of PTA deficiency are described. Clinically, this disorder manifests itself as a tendency to severe bleeding following surgical procedures but not leading to "spontaneous" hemorrhages. In two female patients, the laboratory findings were prolonged clotting time and normal plasma but abnormal serum prothrombin times. This pattern, provided that the presence of a circulating anticoagulant is ruled out, is almost characteristic for PTA deficiency, when found in female persons. In our third patient, a man, the PTA deficiency could only be demonstrated by the sensitive thromboplastin-generation test. PTA appears to be a definitive plasma constituent, essential for the formation of complete blood thromboplastin. Some investigations concerning its physicochemical properties are described.

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Control of Neoplastic Effusion by Phosphoramide Chemotherapy

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The effectiveness of triethylene thiophosphoramide in the treatment of human neoplastic disease was first reported by Shay and his co-workers in 1953.[1] The observations of this group were largely confined to the leukemias and the lymphomas. In a series of 99 patients with various types of far-advanced cancer treated with triethylene thiophosphoramide, palliation of varying degree was achieved, especially in mammary and ovarian carcinoma.[2] Local injection not only permitted larger total doses of drug than did systemic administration but also produced greater regression of local disease, in addition to the effect on distant disease. This report concerns the results of intracavitary injection of phosphoramide agents on serous effusion in a small group of so-called hopeless cancer patients.

N,N′,N″-triethylene thiophosphoramide * crystals were dissolved in sterile distilled water, 10 mg. per cc., and filtered through a sintered glass or a Seitz filter. Solutions of N-(3-oxapentamethylene) N′,N″-diethylene phosphoramide * were prepared in the same way, except that 20 mg. was dissolved in 1 cc. of water. These solutions were stored at refrigerator temperature and were administered undiluted into the pleural, abdominal, or pericardial cavity after withdrawal of fluid. Paracentesis and thoracentesis were performed with a 16- or 18-gauge needle, to permit instillation of the drug without loss immediately following the tap. Triethylene thiophosphoramide was given in doses of 10 to 30 mg.; oxapentamethylene, in doses of 15 to 60 mg. The smaller doses were employed in the presence of leucopenia secondary to previous therapy. Because of the complete absence of clinical side-effects, the procedure was performed in the clinic or office unless the patient was already in the hospital. No particular policy was followed with regard to positioning of the patient following the tap and instillation of the drug.

Twenty-one patients with serous cavity fluid accumulation secondary to metastatic carcinoma were treated with triethylene thiophosphoramide, and oxapentamethylene was employed in 2 cases of mammary cancer. The number of injections per patient per site is given in Table 1.

TABLE 1.—*Total Number of Injections of Phosphoramide According to Site* *

Pleural		Abdominal		Pericardial	
Patients, No.	Injections, No.	Patients, No.	Injections, No.	Patients, No.	Injections, No.
4	1	2	1	1	1
3	2	2	2	..	..
4	3	1	3	..	..
4	4	2	4	..	..
1	8	1	7	..	..
..	..	1	14	..	..

* One patient with ovarian carcinomatosis received injections by two and another by three routes.

The number of cases according to primary site of disease and the results of therapy are given in Table 2. The best results were obtained in the patients with mammary and ovarian carcinoma. In 10 patients pleural fluid reaccumulation has been controlled for one to nine months. Three patients were able to resume work, and seven others carried on partially restricted activity for months. Decreased ascitic fluid reaccumulation was

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TABLE 2.—*Results of Phosphoramide Injection into Serous Cavities*

Primary Site of Cancer	Patients, No.	Fluid Site	Patients, No.	Controlled		Decreased Fluid	No Improvement
				No.	Duration, Mo.		
Breast	13	Pleural	11	8	1-8 (Av. 3)	..	3*
		Ascitic	2	..	...	2	..
Ovary	4	Pleural	2	2	4-9 (Av. 6.5)	..	..
		Ascitic	3	1	5	2	..
		Pericardial	1	1	1½	..	..
Stomach	1	Ascitic	1	..	...	..	1
Lung	1	Pleural	1	..	...	1	..
Malignant hemangiopericytoma	1	Ascitic	1	..	...	..	1
Osteogenic sarcoma	1	Pleural	1	..	...	..	1
Primary lesion unknown	2	Pleural	1	..	1	..	..
		Ascitic	1	..	...	..	1

* Two died in four and five weeks, respectively; in one, pleural space was filled with fibrin clot.

achieved in two patients each with mammary and ovarian carcinoma; in one there was no recurrence for five months. In one case of ovarian carcinoma, triethylene thiophosphoramide was injected intrapericardially after the withdrawal of 1200 cc. fluid, which contained cancer cells. At postmortem examination, six weeks later, there was no evidence of reaccumulation and the pericardium showed no gross evidence of cancer.

In one patient with pleural effusion secondary to bronchogenic carcinoma, chemotherapy was instituted in the hope of controlling fluid reaccumulation so that irradiation therapy of the primary tumor could be given. This was partially accomplished, but the patient died of intercurrent infection. With the exception of control of pleural effusion in one patient with an unknown primary (possibly ovarian) tumor, the re-

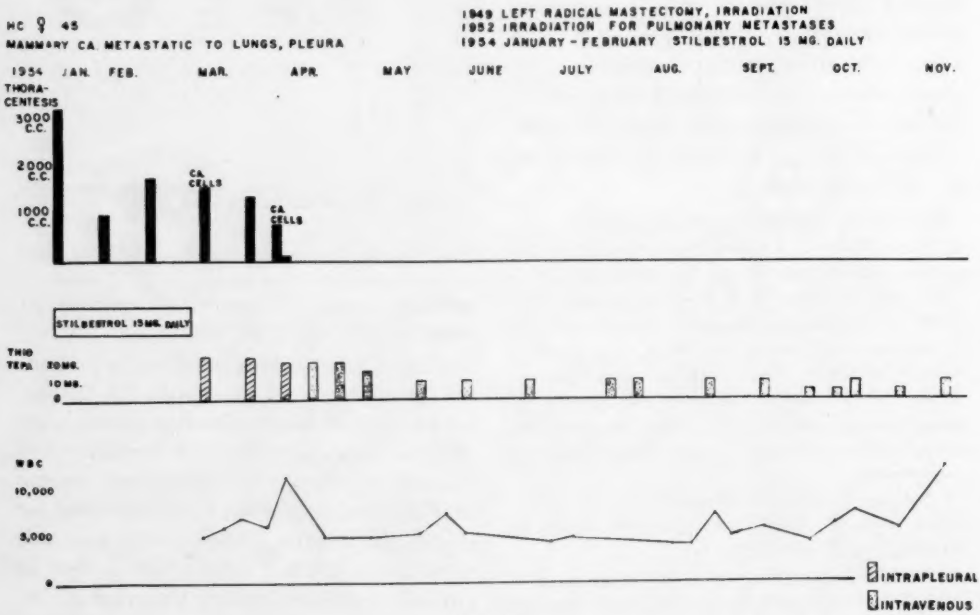

Chart 1.

TABLE 3.—*Follow-Up on Patients Treated with Intracavitary Injection of Phosphoramides*

Duration, Mo.	No. Dead	No. Surviving
1-2	10	..
3	2	2
6	1	1
7	1	2
8	..	1
9	2	1
Total	16	7

viving, 3, 6, 7, 8, and 9 months after intracavitary injections of phosphoramides, have all resumed partial to full activity.

Chart 1 shows the course of a 45-year-old woman who had a radical mastectomy in 1949 for mammary carcinoma, irradiation for pulmonary metastases in 1952, and diethylstilbestrol for two months in 1954 for recurrent disease. There was x-ray evidence of pulmonary metastases and pleural effusion; the patient was completely incapacitated by dyspnea and cough. Cell blocks of pleural fluid showed cancer cells. After three intrapleural injections of 25, 25, and 20 mg. of triethylene thiophos-

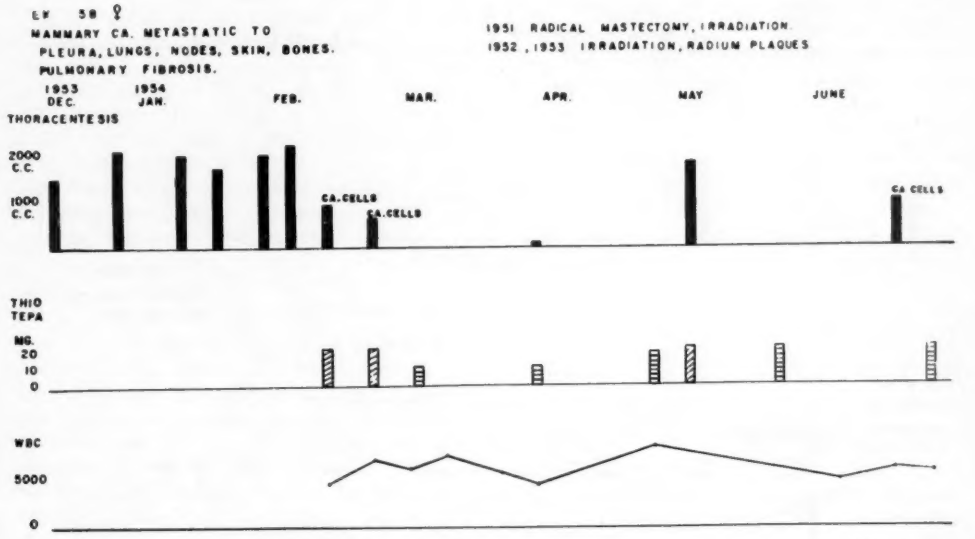

Chart 2.

sults in other cases were poor in this limited series.

Table 3 gives the duration of follow-up in this group of patients. With the exception of one patient who developed bloody pleural effusion containing neoplastic cells four years after a mastectomy, all the other patients had gross evidence of widespread disease. It is understandable therefore that 10 were dead within two months of beginning therapy. The four patients who lived 6, 7, 9, and 9 months, respectively, were able to carry on most of their household duties until shortly before death because the complication of fluid reaccumulation had been controlled. The seven patients who are surphoramide, there has been no recurrence of fluid for eight months. The patient has continued to receive 5 to 20 mg. of drug at one- to three-week intervals, by the intravenous route. The pulmonary lesions are beginning to show roentgenographic evidence of regression, and the patient has returned to part-time work.

E. K. (Chart 2), a 58- year-old woman had a radical mastectomy and postoperative irradiation in 1951, repeated courses of irradiation, and application of radium plaques in 1952 and 1953. When first seen she had recurrent skin nodules, roentgen evidence of metastases to lung and pleura, and rapidly recurring pleural effusion containing cancer cells. Intrapleural injections of triethylene thiophosphoramide controlled the effusion for two- to three-month periods. Injections into recurrent skin nodules resulted in partial regression. The patient

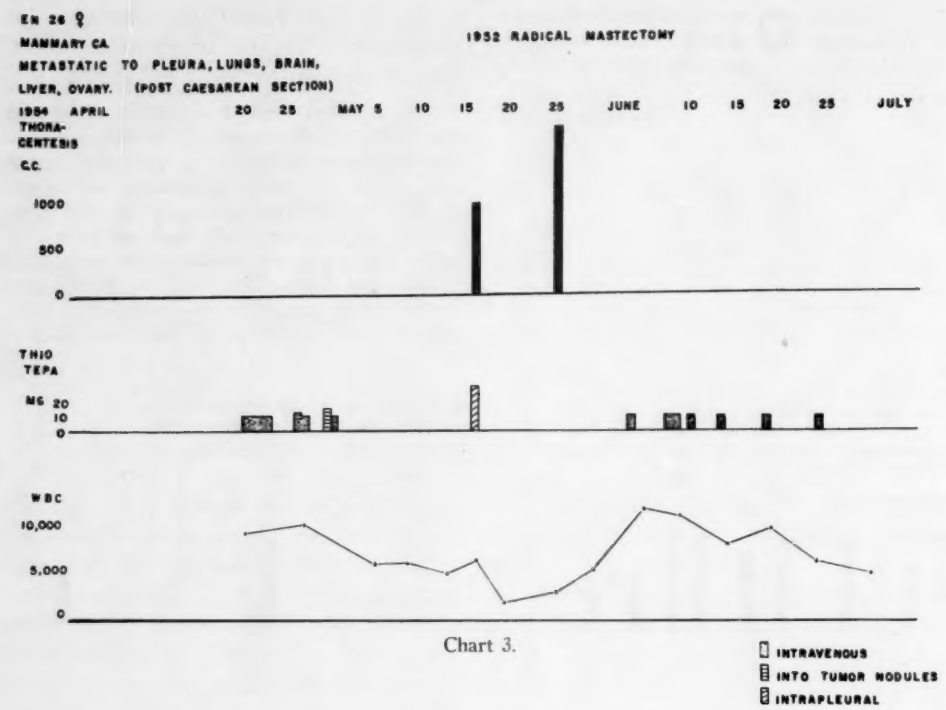

Chart 3.

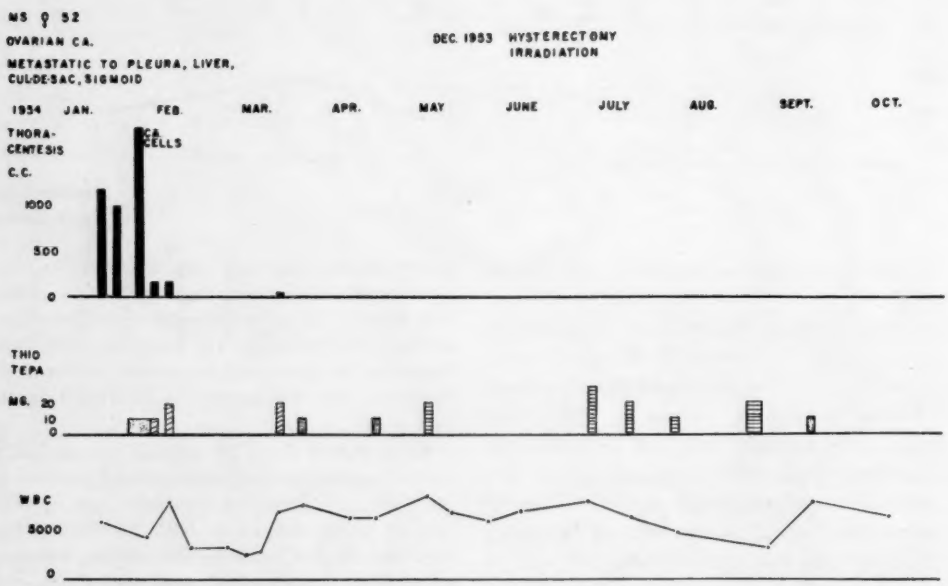

Chart 4.

continued ambulatory on this regime for five months. At the end of this period, she applied for admission to another hospital, where, after one month of hormone therapy, she died.

That pleural fluid can accumulate during or after phosphoramide therapy is demonstrated by Chart 3.

E. N., a 26-year-old woman, had a radical mastectomy in 1952. At the time a caesarian section was performed, in April, 1954, there was demonstrable evidence of metastases to brain, lungs, liver, ovary, and placenta. Intravenous injection of triethylene thiophosphoramide resulted in regression of the signs and symptoms of cerebral metastases. Injection of a nodule on the left breast (biopsy of which showed carcinoma) resulted in disappearance of the nodule. Pleural fluid developed. One intrapleural injection of 25 mg. triethylene thiophosphoramide was followed by a drop in WBC to 1700. Nine days after the first chest tap another thoracentesis was performed; the patient was then fluid-free for one month. Subsequently a febrile course developed, and the patient died with bacteremia. She had not received antibiotics.

Chart 4 demonstrates control of pleural effusion in a case of ovarian carcinoma.

M. S., a 52-year-old woman, at laparotomy in December, 1953, was found to have bilateral ovarian carcinoma with metastases to liver, cul de sac, and sigmoid. One month later there was rapidly recurring bloody pleural effusion containing cancer cells. Three intravenous injections of triethylene thiophosphoramide were followed by intrapleural injection of drug. One month later a tap was productive of only a few cubic centimeters of fluid. Triethylene thiophosphoramide was again injected. Subsequently the patient was treated intravenously as well as by injection into palpable pelvic masses. There was no further demonstrable fluid reaccumulation. The patient returned to her home and part-time activity for six months. She died from intestinal obstruction eight and one-half months after beginning therapy.

Two patients were treated who had had prior therapy with colloidal gold.

A 63-year-old woman with a history of radical mastectomy and postoperative irradiation in 1950, irradiation to the spine in January, 1953, and instillation of radioactive gold in the right pleural space in August, 1953, showed signs and symptoms of pleural effusion in April, 1954. A right pleural tap was productive of only 30 cc. serosanguinous fluid. Thirty milligrams of oxapentamethylene were injected, with no improvement. At postmortem examination six weeks later, the right pleural space was filled with a large fibrin clot and there was collapse of the right lung.

The second patient was a 54-year-old woman who had had a radical mastectomy followed by irradiation in 1948. Ascites developed in 1951, and exploratory laparotomy revealed many tumor nodules. Intra-arterial nitrogen mustard and chlortetracycline (Aureomycin) controlled the ascites for approximately eight months. Subsequently there was a rapid fluid reaccumulation, which another course of nitrogen mustard, followed by irradiation, controlled for only a short period. Subsequent administration of radioactive colloidal gold was followed by an even shorter remission (two to three weeks). The patient required paracentesis every two weeks for many months. A course of intravenously administered triethylene thiophosphoramide was followed by repeated intra-abdominal injection of the drug. Although numerous skin nodules present at the beginning of chemotherapy gradually regressed, fluid reaccumulation stopped only after eight months of therapy. The patient died six weeks later. At postmortem examination the intestines were bound together and covered by fibrin adhesions and the serosa had a peculiar brownish color. Deep inside the mass tiny tumor nodules were found.

COMMENT

The better results obtained in the pleural effusion group as compared to the ascites group may be misleading, owing to the limited number of cases in the latter. However, it is possible that a greater concentration of drug was obtained at the site of the tumor nodules in the pleural effusion cases. Willis [3] states that in the pleural cavity transplanted tumor nodules may be found in all parts but are often most abundant in the costophrenic recesses, on the surfaces of the diaphragm, or along the paravertebral gutter. The ambulatory state of most of the patients in this study would favor concentration of drug at these sites. Although the earliest or most abundant tumor deposits in the peritoneal cavity are often found in the pelvis, no part of the peritoneal surface is exempt, and in advanced disease heavy confluent tumor deposits are almost universally present.[3] The opportunity for uniform contact between drug and tumor would seem less favorable in the abdominal cavity than in the pleural space.

The frequency with which effusion occurs in one or more of the serous cavities as a complication of progressive neoplastic disease cannot be definitely determined. Clark reports an incidence of 29% in 266 consecutive autopsies of men who had malignant disease.[4] Radioactive gold has been used by Seaman and co-workers [5] in 74 patients, with palliation achieved in 50% of both pleural and peritoneal effusions secondary to cancer. Kent and his co-workers [6] report favorable results in approximately one-half of a group of patients treated by intracavitary instillation of radioactive colloidal gold for control of malignant effusions. The criterion for excellent results was the control of fluid by one treatment.

Radioactive gold appears to have two advantages over chemotherapy, namely, complete control of fluid by fewer treatments and effectiveness in a wider spectrum of tumors. Apparently the mode of action is a nonspecific effect resulting from the production of fibrous thickening of the serosa with deposition of fibrin on the surface.[7] These anatomical changes may limit the effectiveness of intracavitary administration of phosphoramides when the latter follows radioactive gold instillation. The poor results obtained in two patients in this study who had been pretreated with radioactive colloidal gold suggest that the drug was unable to penetrate the fibrotic serosa adequately and reach underlying tumor nodules. Systemic therapy in these cases might have been preferable to intracavitary injection.

The mode of action of the phosphoramides in not known. Triethylene thiophosphoramide has produced gross regression and pathologic changes in soft tissue metastases from mammary and ovarian cancer.[2] Effusions associated with mammary and ovarian carcinomatosis responded better to phosphoramide therapy than did effusion associated with other types of cancer in the patients herein reported. It is possible that the phosphoramides act directly on serosal tumor implants. If this is true, the need for multiple injections is understandable, since, like other polyfunctioning alkylating agents, the phosphoramides may act only on actively dividing cells.

It is recognized that continued therapy with phosphoramides by other routes may have been a factor in control of fluid reaccumulation in some of our patients. Such therapy was necessitated by the widespread nature of disease in the patients under study. However intravenous and intratumor therapy alone in one patient (Chart 3) did not prevent development of pleural effusion. In the single patient in the group whose only manifestation of metastatic mammary carcinoma was pleural effusion, there has been no recurrence of fluid for seven months after one intrapleural injection of triethylene thiophosphoramide.

The ease of intracavitary administration of the phosphoramides is of considerable importance, especially in areas in which facilities for use of radioactive materials are not available. Absence of clinical side-effects permits the use of these agents without hospitalization of the patient. The apparent absence of changes in nontumor tissue following phosphoramide therapy is a factor for consideration when indefinite prolongation of life may be anticipated. More extended studies are needed to define the spectrum of tumors in which these agents are effective.

SUMMARY

Twenty-one patients with serous cavity effusions were treated by intracavitary instillation of N,N′N″-triethylene thiophosphoramide, and 2, with N-(3-oxapentamethylene)N′,N″-diethylene-phosphoramide.

Control of pleural effusion lasting from one to nine months was achieved in 10 of 17 patients with mammary and ovarian cancer. Pericardial and ascitic effusion secondary to ovarian cancer was controlled in one patient, and decrease in ascites occurred in four other patients.

In one patient each with carcinoma of the lung and stomach, malignant hemangiopericytoma, and osteogenic sarcoma and in two with unknown primary lesions there was little or no control of effusions after phosphoramide injection.

Response to therapy appears to be related to the primary site of tumor responsible for the effusion.

Dr. Theodore Winship gave encouragement and help in this work.

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Local Application of Epinephrine to Carotid Sinus

Lowering of Blood Pressure and Modification of Hyperreactor Response in Hypertensive Patients

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Research in hypertension has been centered on the role of humoral influences and of increased sympathetic vasomotor tone. The increased sympathetic outflow in hypertension is attributed by some investigators to an abnormality in the hypothalamic circulatory centers or to a hyperirritable vasomotor center which responds to various stimuli by excessive vasoconstrictor impulses.* It seems unusual that research on the role of the sinoaortic regulatory mechanism has not advanced at a similar pace. Why this sensitive mechanism fails to control hypertension has not been thoroughly investigated. The questions of whether an increased sympathetic vasomotor tone is the result of a disturbed inhibitory function rather than primarily central in origin and whether this mechanism can somehow be altered in the hypertensive patient to again reduce the blood pressure to normal levels and to modify the hyperreactor response is completely unanswered. It is our intention to attempt to answer this question.

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From the Cardiovascular Laboratory, Department of Medicine, Chicago Wesley Memorial Hospital and Northwestern University Medical School.

* References 1-3.

Certain fundamental concepts of the physiology of the sinoaortic buffer mechanism must be clearly understood. Normal blood pressure results largely from constant inhibition by the buffer nerves of the physiologically high-central sympathetic outflow.[4] Interruption of buffer nerve activity leads to elevation of blood pressure and tachycardia. Increase of buffer nerve activity leads to lowering of blood pressure and bradycardia. Heymans and others have shown that local application of epinephrine to the exposed carotid sinus and aorta in experimental animals gradually lowers blood pressure, while application of benzazoline (Priscoline), papaverine, and potassium chloride increases it.† These experiments demonstrate that the state of contraction of the arterial wall of the carotid sinus and aorta influence its sensitivity; the more contracted, the more sensitive it becomes. The state of contraction of the large arteries is probably controlled by the sympathetic nervous system. The carotid sinus in dogs has been demonstrated to be controlled, at least in part, by sympathetic fibers originating in the superior cervical ganglion.[7] This control is mediated by release of arterenol (Nor-epinephrine) in the carotid sinus wall and possible release of epinephrine from chromaffin cells in the carotid body and in the arterial wall.

The experiments of Hauss and co-workers showed that the receptors of the buffer nerves are stimulated by the stretching of the arterial wall.[8] This occurs in the arterial system by pulsatile expansion of the wall. One of us (P. K.) conducted experiments which

† References 5 and 6.

have showed that in a contracted state the pulsatile expansion is not diminished, but often is increased, and that in a relaxed state the pulsatile expansion is decreased.[9] The same was suggested by Wiggers and Wegria for the aorta after intravenous epinephrine administration.[10] Other authors have shown that the distensibility of the aorta and carotid arteries while in a contracted state is increased within the range of physiological blood pressure levels, while at low pressures it is decreased.‡ Since we know that the baroreceptors are stimulated by stretching or pulsatile expansion, it is difficult to understand how they would be stimulated more after application of epinephrine to the arterial wall if only tension and resistance to stretch were increased and distensibility, stretching, or pulsatile expansion decreased, as proposed by Heymans and Delaunois.[13] Understanding the histological structure of the elastic arteries aids in reconciling these seemingly opposite points of view. Benninghoff [14] and Elias [15] showed that each smooth muscle fiber of the arterial wall is connected to two elastic fibers. Contraction of the arterial wall is accomplished by tension on the elastic fibers, actually causing them to become curled up. Thus, in a contracted state, there is maximum chance of pulsatile expansion. In the relaxed state, tension is released from the elastic fibers, allowing them to extend smoothly around the circumference of the wall. Thus there is little opportunity for pulsatile expansion in the relaxed arterial wall.

Considering the known physiology, we arrived at the theoretical conclusion that the failure of the sinoaortic regulatory mechanism to control hypertension may be the result of decreased pulsatile expansion of the arterial wall caused by relaxation and distension of the stretch-sensitive areas, resulting in less stimulation of the pressoreceptors. This in turn allows a greater central sympathetic outflow, resulting in hypertension. By local application of epinephrine the carotid sinus wall would be contracted, its tension increased, and the normal pulsatile expansion restored, which would result in more inhibition of the central sympathetic outflow, and the blood pressure would be lowered.

The increased sympathetic outflow in prehypertensive and hypertensive patients has been demonstrated by the hyperreactor response to the Valsalva maneuver by Wilkins [16] and others. The mechanism of the Valsalva maneuver has been adequately explained by other authors.§ The maneuver is usually analyzed in four phases. We have confined our observations to Phase 4, measuring the overshoot and subsequent bradycardia.‖

The work of McAfee and co-workers demonstrated that the location of the carotid sinus in man is relatively constant.[25] Previous experience of one of us (P. K.) in procaine block of the carotid sinus in man had demonstrated that local application of drugs to the carotid sinus can be done with considerable certainty.[26] We therefore decided to attempt to change the carotid sinus function in human hypertensive subjects by the local application of epinephrine.

METHODS

The Valsalva maneuver was performed on 20 patients with essential hypertension in different stages of the disease. Twelve normotensive patients were also tested. Four normal patients were used as controls for the epinephrine injection. All pressures were taken with an intra-arterial needle and were recorded by means of an electromanometer. Both carotid sinus arterial walls were infiltrated with 50γ of epinephrine diluted in 2 cc. of isotonic saline. The skin was first infiltrated with procaine. Then, with use of a 1½ in. (3.75 cm.) 24-gauge needle and a direct anterior approach medial to the sterno-

‡ References 11 and 12.

§ References 17-24.

‖ The four phases are (1) initial blood pressure rise when blowing is begun, (2) fall of blood pressure during blowing, (3) precipitous fall of blood pressure at the moment blowing ceases, (4) the recovery periods, including the overshoot and subsequent bradycardia. The overshoot is the rise of blood pressure above resting levels caused by the suddenly increased cardiac output meeting the markedly constricted peripheral arterial system. It is counteracted by the sinoaortic regulatory system which, when functioning properly, causes a sudden peripheral vasodilatation and a bradycardia. Thus, the Valsalva maneuver is a test of the integrity of the sinoaortic regulatory system.

cleidomastoid muscle, the epinephrine was injected directly on the outer wall of the carotid sinus. In order to eliminate the effect of direct stimulation by pressure, 15 minutes were allowed to elapse before blood pressure recordings were taken. The Valsalva maneuver was done once or twice 15 to 30 minutes after injection. The blood pressure by cuff method was followed up to three hours after the injection of epinephrine.

EXPERIMENTAL RESULTS

Effect of Epinephrine Infiltration of Carotid Sinus on Valsalva Maneuver.—Twelve normal persons had an average overshoot of 29/14 mm. Hg. This overshoot reached its maximum in 5.0 seconds and was followed by a bradycardia in all but one; it occurred on an average of 2.6 seconds after the maximum overshoot. The decrease of pulse rate averaged 19% below resting levels. The maximum systolic overshoot was 52 mm., and the maximum diastolic was 44 mm. Hg.

Twenty hypertensives had an average overshoot of 43/29 mm. Hg, which reached a

TABLE 1.—*Response of Normal Patients to Valsalva Maneuver Before Epinephrine Application to Carotid Sinus*

			Resting			After Valsalva Maneuver					
			Blood Pressure, Mm. Hg			Overshoot, Mm. Hg			Bradycardia		
Case	Age, Yr.	Sex	Systolic	Diastolic	Pulse	Systolic	Diastolic	Time *	Pulse	% Decrease	Time †
1	20	F	120	80	80	46	44	3.4	72	10	1.0
2	35	F	120	64	72	10	12	3.4	68	6	4.2
3	19	M	138	70	94	52	22	4.4	64	32	2.3
4	57	M	148	78	88	12	2	7.5	88	..	...
5	20	F	126	78	86	22	0	4.0	64	25	2.0
6	43	F	132	82	86	8	0	10.4	78	9	2.8
7	30	M	110	64	75	46	20	5.0	62	17	1.0
8	20	M	110	52	60	36	22	5.0	42	30	1.0
9	23	M	116	64	100	30	16	3.4	72	28	2.4
10	32	F	90	40	82	50	16	4.6	72	12	6.6
11	16	M	164	60	94	0	0	3.4	68	28	1.0
12	24	M	110	60	62	32	8	6.0	50	19	4.6
Average			119	66	81	29	14	5.0	67	19	2.6

* Time of maximum overshoot was measured from the end of blowing.
† Time of maximum bradycardia was measured from the maximum overshoot to the beginning of bradycardia persisting for at least six seconds.

TABLE 2.—*Response of Normal Patients to Valsalva Maneuver after Epinephrine Application to Carotid Sinus*

			Resting			After Valsalva Maneuver					
			Blood Pressure, Mm. Hg			Overshoot, Mm. Hg			Bradycardia		
Case	Age, Yr.	Sex	Systolic	Diastolic	Pulse	Systolic	Diastolic	Time *	Pulse	% Decrease	Time †
1	20	F	116	66	86	40	24	3.8	74	14	1.0
2	35	F	120	70	64	10	8	3.8	80	..	...
3	19	M	140	80	86	50	20	6.4	70	19	2.5
4	57	M	110	58	81	4	0	7.0	81	..	...
Average			121	68	79	26	13	5.2	76	17	1.8

* Time of maximum overshoot was measured from the end of blowing.
† Time of maximum bradycardia was measured from the maximum overshoot to the beginning of bradycardia persisting for at least six seconds.

The Valsalva maneuver was performed by having the patient blow against the resistance of an aneroid manometer maintaining an intra-oral pressure from 40 to 50 mm. Hg for 15 seconds.[17]

The results of the Valsalva maneuver before and after epinephrine infiltration of the carotid sinus were analyzed and compared with data of the normal control subjects.

TABLE 3.—*Response of Hypertensive Patients to Valsalva Maneuver Before Epinephrine Application to Carotid Sinus*

				Resting			After Valsalva Maneuver					
				Blood Pressure, Mm. Hg			Overshoot, Mm. Hg			Bradycardia		
Case	Age, Yr.	Sex	Duration of Hypertension	Systolic	Diastolic	Pulse	Systolic	Diastolic	Time *	Pulse	% Decrease	Time †
1	63	F	2	240	106	84	40	54	3.6	66	21	8.8
2	65	F	25	210	100	72	30	10	5.4	72	..	...
3	57	M	10	250	106	106	0	12	8.0	106	..	...
4	70	F	8	200	100	88	24	10	7.0	88	..	...
5	51	F	7	190	90	88	60	35	5.4	80	9	5.0
6	60	F	3	210	90	62	50	30	7.4	70	..	...
7	44	F	2	230	110	65	90	100	2.0	60	8	15.0
8	57	F	13	200	76	83	45	4	7.4	83	..	...
9	44	F	7	240	120	86	30	34	8.8	86	..	...
10	54	F	7	170	90	72	45	30	6.0	72	..	...
11	51	M	8	210	130	93	70	62	4.2	80	14	2.6
12	48	F	2	210	100	60	60	20	6.2	60	..	...
13	46	F	2	200	100	80	65	35	6.2	70	12	4.6
14	66	F	15	210	100	80	26	18	9.2	80	..	...
15	42	M	½	180	100	100	60	38	3.5	75	25	1.5
16	50	F	2	190	100	79	60	20	5.8	65	18	11.6
17	46	F	10	190	105	105	0	0	10.2	105	..	...
18	66	F	8	210	90	90	25	15	7.8	90	..	...
19	68	F	15	200	100	86	50	25	4.6	86	..	...
20	44	M	20	250	130	100	20	28	4.0	100	..	...
Average.....				209	102	84	43	29	6.1	80	15	7.0

* Time of maximum overshoot was measured from the end of blowing.
† Time of maximum bradycardia was measured from the maximum overshoot to the beginning of bradycardia persisting for at least six seconds.

TABLE 4.—*Response of Hypertensive Patients to Valsalva Maneuver After Epinephrine Application to Carotid Sinus*

				Resting			After Valsalva Maneuver					
				Blood Pressure, Mm. Hg			Overshoot, Mm. Hg			Bradycardia		
Case	Age, Yr.	Sex	Duration of Hypertension	Systolic	Diastolic	Pulse	Systolic	Diastolic	Time *	Pulse	% Decrease	Time †
1	63	F	2	204	90	66	74	48	3.8	66	..	...
2	65	F	25	160	68	66	20	8	4.4	66	..	...
3	57	M	10	146	60	90	14	20	4.0	70	22	1.0
4	70	F	8	124	60	70	4	0	6.0	75	..	...
5	51	F	7	160	78	78	40	32	5.8	78	..	...
6	60	F	3	120	60	62	30	10	8.0	52	16	3.6
7	44	F	2	125	60	58	45	20	6.0	52	10	15.0
8	57	F	13	128	50	68	42	6	6.6	65	4	3.0
9	44	F	7	140	60	60	20	15	6.0	60	..	...
10	54	F	7	160	80	72	40	20	6.8	72	..	...
11	51	M	8	182	108	79	58	26	6.2	65	18	1.6
12	48	F	2	200	80	68	20	10	5.0	64	6	2.2
13	46	F	2	162	78	80	83	32	6.2	60	25	4.0
14	66	F	15	180	84	76	50	21	7.5	65	14	3.0
15	42	M	½	122	60	70	48	22	2.4	60	..	...
16	50	F	2	120	70	68	50	20	7.8	61	10	6.8
17	46	F	10	132	70	100	0	0	7.0	79	20	2.2
18	66	F	8	190	90	80	10	0	9.2	80	..	...
19	68	F	15	160	80	88	40	28	5.0	75	15	10.4
20	44	M	20	220	116	94	20	14	3.2	100	..	...
Average.....				157	75	75	35	18	5.8	68	14	4.8

* Time of maximum overshoot was measured from the end of blowing.
† Time of maximum bradycardia was measured from the maximum overshoot to the beginning of bradycardia persisting for at least six seconds.

maximum in 6.1 seconds. The maximum systolic overshoot was 90 mm. Hg, and the maximum diastolic, 100 mm. Hg. This was followed by bradycardia in only seven patients (35%). This bradycardia occurred in an average of 7.0 seconds after the maximum overshoot. In those patients who did exhibit bradycardia there was a 15% decrease of pulse rate below resting levels. The average duration of hypertension in patients who exhibited bradycardia following the Valsalva maneuver was 3.3 years, compared with the average duration of 11 years in those in whom no bradycardia occurred.

After local infiltration of the carotid sinus, as previously described, the hyperreactor response was modified in the hypertensive patients and essentially unchanged in the four normal controls. In 20 hypertensive patients the average overshoot was 35/18 mm. Hg. The maximum systolic overshoot was 83 mm. Hg, and the maximum diastolic was 48 mm. Hg. The overshoot occurred in 5.8 seconds. It was followed by bradycardia in 11 of 20 patients (55%). The bradycardia occurred an average of 4.8 seconds after the maximum overshoot. The average duration of hypertension of the 11 patients exhibiting bradycardia was 7.5 years, as compared with 9.4 years in those not having bradycardia. In those patients developing bradycardia, the average decrease in rate below resting levels was 14%.

Effect of Epinephrine Infiltration of Carotid Sinus on Blood Pressure.—After local infiltration of the carotid sinus with epinephrine, blood pressure was decreased in all hypertensive patients. Only slight change in blood pressure occurred in two of the four normal controls. In 9 of the 20 hypertensive patients the blood pressure decreased to normal levels. The reduction in blood pressure in some instances remained as long as three hours. The average duration of hypertension in patients whose blood pressures decreased to normal levels was 5.2 years, as compared with 10 years in those whose pressures did not return to normal. The average decrease in systolic pressure was 52 mm. Hg, and average decrease in diastolic pressure was 27 mm. Hg. In seven patients with a known duration of hypertension of three years or less the average fall was 57/29 mm. Hg. In 13 patients with a duration of hypertension of more than three years, the average fall was 49/26 mm. Hg. In addition, the resting pulse rate decreased in injected hypertensive patients. The average pulse rate was 84 before and 75 after injection.

TABLE 5.—*Average Results of All Groups*

	Resting			After Valsalva Maneuver					
	Blood Pressure, Mm. Hg			Overshoot, Mm. Hg			Bradycardia		
	Systolic	Diastolic	Pulse	Systolic	Diastolic	Time *	Pulse	% Decrease	Time †
Normals before epinephrine.....	119	66	81	29	14	5.0	67	19	2.6
Normals after epinephrine.....	121	68	79	26	13	5.2	76	17	1.8
Hypertensives before epinephrine	209	102	84	43	29	6.1	80	15	7.0
Hypertensives after epinephrine..	157	75	75	35	18	5.8	68	14	4.8

* Time of maximum overshoot was measured from the end of blowing.
† Time of maximum bradycardia was measured from the maximum overshoot to the beginning of bradycardia persisting for at least six seconds.

COMMENT

The experimental results have some limitations which must be considered. First, the number of cases is not large, and so conclusions would perhaps be subject to some variation if larger numbers were used. The experimental results certainly must be influenced by the amount of epinephrine actually reaching the carotid sinus. This, of course, was variable, since the injection, of necessity, must be done blindly. No way is available to modify the action of the aortic buffer nerves under the conditions of this experiment. Finally, there are other reflexes in-

volved in the Valsalva maneuver which influence response and might change the effect of epinephrine on the carotid sinus reflex.

Despite these limitations our results indicate that there is a difference in reaction of the sinoaortic regulatory system in normotensive and hypertensive persons in response to the Valsalva maneuver. The average overshoot in hypertensives is greater and occurs later than that seen in normals. All but one normal (92%) developed bradycardia, whereas only 35% of hypertensives developed bradycardia, and this takes more than twice as long to occur as in normals. The overshoot in the Valsalva maneuver is attributed to the combined effect of the suddenly increasing cardiac output meeting the peripheral vasoconstriction, which occurs reflexly in the phase of forced expiration. A well-functioning carotid sinus and aortic mechanism prevents abnormally high overshoots by reflex bradycardia and peripheral vasodilatation. In hypertension this function of the pressoreceptors is apparently faulty. Application of epinephrine to the carotid sinus definitely altered the hyperreactor response in hypertensive patients. Blood pressure was lowered in all hypertensive patients. This lowering of pressure was definite and persisted for periods of time up to three hours. Only slight effect on blood pressure and overshoot was noted in the normal controls.

There is little doubt, then, that the local application of epinephrine to the carotid sinus in hypertensive patients will both modify their hyperreactor response and lower their blood pressure. Increased sympathetic vasoconstrictor outflow in hypertension can thus be abolished by this procedure. This tends to bear out the hypothesis that increased sympathetic tone in hypertension may be due to decreased inhibition of the normal physiological sympathetic outflow rather than caused by primary disturbance of the vasomotor or sympathetic center. Our experiments suggest that this decreased inhibition in hypertension is the result of a decreased elasticity and pulsatile expansion of the carotid sinus due to abnormal relaxation and dilatation. Local application of epinephrine results in contraction, increased tension, and normal pulsatile expansion of the dilated arterial wall. Thus, the sympathetic outflow will be decreased to normal by the baroreceptors. Our results tend to show that the shorter the duration of hypertension the better the response to the epinephrine application. In early cases, therefore, this defect is reversible by local epinephrine application. In more advanced cases, however, secondary changes maintain the elevated blood pressure and also prevent response to epinephrine. Finally, it should be pointed out that, although this experiment opens new aspects to better understanding of the pathogenesis of essential hypertension, no therapeutic implications are suggested.

CONCLUSIONS

Patients with hypertension had an exaggerated response to the Valsalva maneuver, and only 35% of them developed subsequent bradycardia, as compared with 92% of the normals studied.

Local application of epinephrine to the carotid sinus of hypertensive patients modified their hyperreactor response as measured by the overshoot in the Valsalva maneuver.

Local application of epinephrine to the carotid sinus lowered blood pressure in all hypertensive patients studied by a significant amount for periods lasting up to three hours.

It is possible that the increased sympathetic outflow in essential hypertension is caused by an impaired buffer nerve mechanism due to dilatation, abnormal relaxation, and decreased pulsatile expansion (loss of elasticity) of the barosensitive arterial walls. This may be a fundamental defect in essential hypertension.

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Contribution to Study of Wilson's Disease

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We regard it of interest to report our study of three brothers affected with Wilson's disease, in two of whom the process appeared with its most characteristic clinical picture and in one of whom the only finding in relation to this disease was the presence of amino-aciduria.

The nature of the hepatolenticular degeneration that appears in Wilson's disease is yet unknown, but in the last few years, by evaluation of the disturbances of the metabolism of copper and the amino acids eliminated in the urine, new concepts have been brought forth which are worthy of further research.

Baker,[1] in 1948, summarized the theories which up to that time had tried to explain the nature of Wilson's disease as follows: 1. It is a degenerative process affecting the liver and brain. 2. It is an acquired disease, and both organs are damaged simultaneously by an endogenous toxin. 3. The primary lesion is found in the liver, and the brain is affected secondarily.

The fact that the pathologic process affects the liver and the brain simultaneously in Wilson's disease, such a concomitant affection being infrequent in other types of cirrhosis, even in the nodular toxic type, as well as the appearance of this disease in members of the same family, lead us to consider that the pathogenic mechanism of this disease has some interesting peculiarities.

Attempts have been made to explain the manifestations of Wilson's disease on the ground of an abnormal storage of copper within the tissues. It is known that copper and other heavy metals are powerful inhibitors of enzymatic activity even at high dilutions. Lipmann [2] found that copper at a dilution of 0.636 mg. per ml. would inhibit glycolysis in muscle, and if we consider that in certain cases the concentration of copper in the basal ganglia may be higher than 1 mg. per 100 gm. we can deduce that this metal can interfere with the respiratory process of tissues by paralyzing enzymatic systems, thus producing necrosis and excavation of the basal ganglia.

It has been proved that experimental cirrhosis can be produced by the administration of sufficient amounts of copper or manganese, but this is definitely not an explanation for the pathogenesis of Wilson's disease, as it cannot be considered that simple intoxication by copper would originate the disease. However, it is interesting to determine why copper is retained and stored in the body. The simplest explanation would be that the primary liver damage in Wilson's disease would interfere in the elimination of this metal by the bile. However, in other, more intense forms of cirrhosis, with a greater affection of the excretion of bile, there is no retention of copper. Denny-Brown and Porter determined the elimination of copper through bile in three patients with hepatolenticular degeneration, finding normal values instead of the expected reduction. This could be explained by an increased reabsorption, but the investigation with labeled copper has not yet been conclusive. The abnormal metabolism of copper may be presumed to be, in these patients, secondary to other severer disorders. It is known that in the final stages of serious hepatic insufficiencies, certain amino acids, most frequently leucine and tyrosine, appear in the urine. When the liver

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function fails, it can no longer accomplish a deaminizing action, thus causing an increase of amino acids in the plasma and subsequently amino-aciduria. Uzman and Denny-Brown [4] disregarded the concept that in the cirrhosis of Wilson's disease the altered liver function brought about by the cirrhotic condition is responsible for the altered metabolism of amino acids. By hepatic biopsy, these authors demonstrated in one patient (of a pseudosclerotic variety) that there were no signs of cirrhosis; thus, the altered amino acid metabolism could not be explained by an hepatic insufficiency. The increased amino-aciduria is a constant finding in these patients, and this has been verified by all observers. In our patients a constant elimination of great amounts of amino acids by the urine has been verified.

Uzman and Denny-Brown consider that in normal subjects the output of amino nitrogen in 24 hours is from 100 to 200 mg. We have found in our cases a daily elimination reaching as high as 1 gm. Studies of Cooper [5] have proved that in Laennec's cirrhosis there is no increase in the output of amino acids, except in the terminal stages. He also determined that in hepatolenticular degeneration the level of amino acids in the plasma is normal (from 3.3 to 5.7 mg. per 100 ml., these data being in accordance with our findings). These facts prove that amino-aciduria in Wilson's disease is primary and that its cause is not the disturbance in the hepatic function nor an increase in the protein catabolism, which would cause an increase in the level of amino acids in the plasma. The amino-aciduria is present before the disturbances of the liver and brain, for it has been found existing in brothers of patients with Wilson's disease, the amino-aciduria being the only abnormal finding in such persons, who are absolutely free from any liver or cerebral disturbance. As an example, the report of Uzman and Hood presents a family in which four brothers out of nine died of Wilson's disease, with a finding of persistent amino-aciduria in the other five brothers, who otherwise showed no symptoms. Likewise, Dent and Harris [7] found increased levels of amino-aciduria in two symptomless brothers of patients with hepatolenticular degeneration. Similar findings have been reported by De Verdier [8] and Hood and Fagerberg.[9] Studying the amino acid excretion in three younger brothers of our patients, we have seen high values of excretion (345 mg. in 24 hours) in the third brother and normal values in the other two younger brothers. Personally, we believe that in the study of the pathogenesis of hepatolenticular degeneration we must consider as the initial disturbance the increased excretion of amino acids by the urine, and the explanation of its cause is most important, because this finding precedes the clinical onset of the disease and is the most outstanding metabolic disturbance once this process has developed completely.

From the study of our patients, we shall try to answer the following questions: 1. What is the cause of amino-aciduria in Wilson's disease? 2. What is the relationship between amino-aciduria and the retention of copper in the tissues and its increased elimination through the urine?

1. SIGNIFICANCE AND CAUSE OF AMINO-ACIDURIA

When the amino-aciduria was first observed as a constant finding in Wilson's disease, attempts were made to establish a similarity between Wilson's disease and the Toni-Fanconi syndrome.

The Toni-Fanconi syndrome is a peculiar metabolic disturbance, apparently a congenital condition, characterized by a high level of excretion of amino acids, glucose, inorganic phosphorus, and bicarbonate in the urine. These patients, being children, show clinical symptoms of rickets or osteomalacia resistent to vitamin D and also an increase of the serum alkaline phosphatase and in some cases a diminution of the alkaline reserve.

The persistent elimination of glucose through the urine leads to a loss of weight and to transitory crises of hypoglycemia, usually occurring in the morning before breakfast.

Polyuria is another symptom that appears, and it may at first be mistaken for the polyuria of diabetes insipidus. Sometimes the increased cystinuria produces a deposition of cystine calculi in the kidney. But undoubtedly the most important fact about the Toni-Fanconi syndrome is that, in a number of patients with this disease, a nodular hepatic cirrhosis may develop after some time (Himsworth [10] and Dent [11]), similar to that appearing in Wilson's disease. The cause of this cirrhosis could be explained by the continuous loss of amino acids, similar to what occurs in experimental cirrhosis caused by a protein deficiency. This, we believe, is a fundamental fact in the pathogenesis of the cirrhosis of hepatolenticular degeneration.

Darmady and his colleagues,[12] in an attempt to find an explanation for the pathogenesis of the Fanconi syndrome, regarded it as a structural disorder of the nephron. In two of their cases, the authors made a careful microdissection of the kidney. Isolating the nephrons, they found that the proximal convoluted tubule was constantly shorter than normal and that it was joined to the glomerulus by a narrow, swan-like neck. Bickel, however, criticises these findings and states that the lesions found by Darmady and his colleagues are not constant and, on the other hand, cannot account for the retention of cystine, a finding that so frequently accompanies this syndrome, which, according to this author, may be due to an acquired lesion. Hottinger [14] and Drabløs [15] did not find any anatomic renal lesion in the study of two children with the Fanconi syndrome.

If no histologic alterations are found in such cases, then we could admit that in the Toni-Fanconi syndrome there is a primary disturbance of the metabolic function of the kidney, i. e., a "disnephria" in accordance with the terminology of Jiménez Díaz.* This biochemical disturbance may perhaps be due to a constitutional mutation, possibly due to a disturbance of the deaminase system, for, according to Ferguson and his colleagues, there is a close parallel between the intensity of renal deaminization and tubular reabsorption of amino acids. Stowens and Dent,[18] on one hand, and Cooke, Barclay, Govan, and Naglay,[19] on the other, maintain that in the Fanconi syndrome there is an absence of phosphatase in the proximal convoluted tubule, which could explain the increased renal elimination of phosphatase, glucose, and certain amino acids which, in the normal subjects, are absorbed as phosphoric esters.

One of the first points that we wanted to elucidate about our patients with hepatolenticular degeneration was whether any of their findings were comparable to those observed in the Toni-Fanconi syndrome.

In Table 1 we see that the renal threshold for glucose is normal in both brothers and that the ammoniopoietic function of the kidney shows an increase in both, this being an index perhaps of the greater substratum that the high level of amino-aciduria supplies to the kidney. In the next table, Table 2, the relevant data of blood and urine are presented in comparison to those in the Toni-Fanconi syndrome. The values of blood phosphorus, calcium, potassium, alkaline reserve, and acid phosphates are normal. Only the values of alkaline phosphatase are increased (48.6 units in the older brother and 62 units in the other). The only similarities between our findings and those commonly observed in Fanconi's syndrome are amino-aciduria, increased formation of ammonia, and high values of alkaline phosphatase. An explanation for the amino-aciduria and subsequent increase ammoniopoiesis will be discussed further on. The increase of alkaline phosphatase may be considered as a sign of a rachitic process in our patients, a form of renal rickets like that found in Fanconi's syndrome. Von Economo and Luthy [20] have described a tendency for osteoporosis in Wilson's disease. However, it is quite contradictory that the values of calcium and phosphorus in the blood are normal, that no other metabolic disturbances are found, and that no signs of bony lesions exist.

On the other hand, in common rickets there is also an increase amino-aciduria. Recently Jonxis and Huisman [21] have con-

* References 16 and 17.

TABLE 1.—*Study of Renal Function*

		Basal	30 Min.	60 Min.	90 Min.	120 Min.	150 Min.
Renal Threshold for Glucose							
J. R.	Glycemia, gm./100 cc.	0.98	1.42	1.47	1.54	1.40	....
	Glycosuria	None	None	None	None	None	None
P. R.	Glycemia, gm./100 cc.	0.83	1.40	1.48	1.28	1.20	1.07
	Glycosuria	None	None	None	None	None	None

		1st Hr.	Minute Volume	2d Hr.	Minute Volume	Urea in Blood, Gm./100 Cc.
Urea Clearance (Van Slyke)						
J. R.	Urine, cc.	204	3.4	280	4.6	0.40
	Urea in urine, gm./100 cc.	7.50	...	9	...	....
	Maximal blood clearance, %	86.06	...	62.10	...	....
P. R.	Urine, cc.	183	2.9	221	3.68	0.40
	Urea in urine, gm./100 cc.	7.85	...	7.25	...	....
	Maximal blood clearance, %	75.69	...	84.65	...	....

	Urine in 24 Hr., Cc.	pH	Acidity Measured in Cc. of NaOH N/10	Ammoniacal N, Mg. in 24 Hr.*	Ammonia, Mg. in 24 Hr.	Amino-Aciduria, Mg. in 24 Hr.
Ammoniopoietic Function						
J. R.	1800	5-6	20	1.302	1.584	321
P. R.	1900	6	19	1.936	2.159	530

* Normal value for ammoniacal nitrogen is up to 820 mg. in 24 hours (Sörensen's method).

firmed this finding in their patients and have observed that excess amino acids eliminated through the urine (these not differing from those which normally appear in the urine) decrease or disappear after a massive dose of vitamin D. We have twice administered massive doses of vitamin D (600,000 units each) to our patients, with an interval of five days, but no variation in the values of alkaline phosphatase or amino-aciduria were found.

In cases of Fanconi's syndrome it has been observed that the rickets is resistant to vitamin D therapy and that, likewise, the amino-aciduria remains unmodified. But in the majority of cases the amino acids which appear in the urine are qualitatively different from those eliminated by normal subjects (when cystine appears we may consider it as a case of the Lignac-Faconi syndrome); however, in our patients there is no qualitative alteration of the amino acids excreted but rather a quantitative one, as we may observe in the Figure, in which the only amino acids almost exclusively eliminated are

TABLE 2.—*Comparison of the Toni-Fanconi Syndrome with Data from Patients J. R. and P. R.*

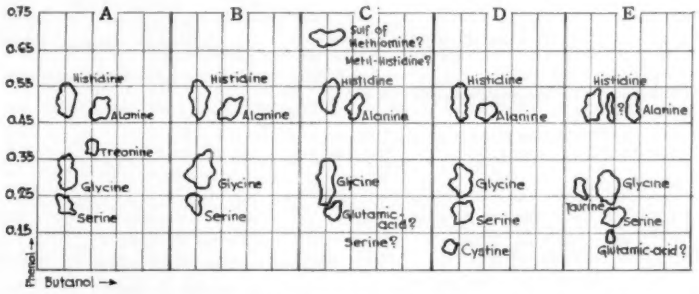
	Normal Values	Toni-Fanconi Syndrome	Patient J. R.	Patient P. R.
Blood				
Calcium, mg./100 cc.	8.2-11.6	Reduced	9.9	9.8
Inorganic phosphorus, mg./100 cc.	2.4-4.4	Reduced	4.2	4.6
Potassium, mg./100 cc.	16-21	Reduced	20	19
Serum acid phosphatase, units/100 cc.	0-1	Normal	1.4	2.6
Serum alkaline phosphatase, units/100 cc.	2.2-8.6	Increased	48.6	62
CO_2-combining power, vol. %	54-66	Reduced	54	57
Glucose, gm./100 cc.	0.690-1.30	Normal	0.98	0.83
Urine (amounts in 24 hr.)				
Glucose	None	Present	None	None
Phosphates (as inorganic phosphorus), gm.	0.320-1.600	Increased	0.298	0.199
Amino acids (in amino N), mg.	0-200	Increased	Increased	Increased
N (as ammonia), gm.	0.410-0.820	Increased	1.584	2.159
Calcariuria, gm.	0.010-0.300	Increased	0.188	0.094
Bone changes by x-rays	.....	Remarkable rarefaction, rickets, osteomalacia	Slight rarefaction	None

histidine, aminoacetic acid (glycine), and serine.

If the high values of the serum alkaline phosphatase cannot be ascribed to a process of latent rickets, we may consider it as another proof of the existance of a degenerative process of the central nervous system, identical to what happens in Friedreich's ataxia and in lathyrism, where we have also found an increase, in certain cases quite considerable, of alkaline phosphatase. In an attempt to eliminate a possible tubulorenal derangement in our patients, we are in the process of acquiring some data by means of a test which consists in administering, during some time, methyltestosterone, determining simultaneously the amino-aciduria; the values of calcium, phosphorus, and potassium in the renal function are concerned and that the cause of the amino-aciduria in Wilson's disease does not lie primarily in the kidney.

2. RELATIONSHIP BETWEEN AMINO-ACIDURIA AND THE RETENTION OF COPPER

According to our present knowledge, there is no doubt that the increased deposition of copper in certain structures of the organism, specially in the basal ganglia, is responsible for the symptoms and neurologic signs of the disease. A proof that favors this contention is the clinical improvement that follows the increased elimination of copper by the kidney under treatment with dimercaprol (BAL), as observed by Cumings,[23] Denny-Brown and Porter,[3] and many other workers who have published more recently. We have likewise confirmed the favorable effects of



Chromatography of amino acids in urine. Techniques used: phenol, Kirby buffer; butanol, acetic acid, water; 20 cu. mm. of urine. Normal value (according to Pope and Stevens [28]) up to 200 mg. of amino nitrogen in 24 hours. *A*, Patient J. R., age 22, total amino-aciduria=321 mg. amino nitrogen; *B*, Patient P. R., age 19, total amino-aciduria=515 mg. amino nitrogen; *C*, Patient E. R., age 17, total amino-aciduria=341 mg. amino nitrogen; *D*, Patient Jua. R., age 14, total amino-aciduria=150 mg. amino nitrogen; *E*, Patient Pi. R., age 11, total amino-aciduria=140 mg. amino nitrogen.

plasma, and the ammoniopoietic function. We have based this test on the observations of Anderson, Miller, and Kenny,[22] who have treated by this means a number of cases of the Fanconi syndrome, having observed that methyltestosterone reduces the excretion of ammonia and amino nitrogen by the kidney and favors the retention of calcium, phosphorus, and nitrogen, causing a hypertrophy of the proximal convoluted tubules.

Taking into consideration the data we have at hand up to the present, we venture to state that hepatolenticular degeneration resembles the Fanconi syndrome only superficially as far as, strictly speaking, disturbances of the dimercaprol on the neurologic symptoms of our patients. The degree of improvement obviously depends upon the intensity of the organic lesions; for if the characteristic excavating lesion in the putamen has already taken place we can expect but minimal beneficial effect from the treatment with dimercaprol. In our cases the improvement has always been more marked in the younger of the two brothers, who was less affected by the disease. We are under the impression that the fatal course of the disease has been greatly retarded in both brothers.

Some workers believe that copper is eliminated by the kidney bound to amino acids.

Matthews, Milne, and Bell [24] affirm that an overdose of lysine and alanine, given orally, produces an increased elimination of these amino acids by the kidney together with a considerable increase in the elimination of copper (from two to five times the normal value), similarly to what occurs when copper is mobilized by the administration of dimercaprol.

However, another series of facts do not agree with the previous assumption. In the first place, the amino-aciduria precedes, in certain cases for many years, the clinical onset of the disease and the increased elimination of copper through the kidney. In our case, the brothers of our patients who did not manifest any symptoms of neurologic or hepatic affection gave normal values of copper in the urine, in spite of the fact that in one of them the amino-aciduria was high (341 mg.). On the other hand, there is no increase in the elimination of copper by the kidney in the Fanconi syndrome.

The data gathered from our patients are sufficient to exclude the possibility that copper is mobilized in the organism (either as it is stored in tissues or as it is eliminated by the kidney) simultaneously or in some way synchronous with the mobilization of amino acids.

In Table 3, we present the values of amino nitrogen in the blood, amino nitrogen in the urine, total nitrogen, and copper in the urine, before and during the treatment with dimercaprol in our first patient. While the values of copper in the urine increase from 164γ (average value before treatment) to 270γ (average value during treatment), the values for amino nitrogen in the blood and in the urine do not vary significantly. In Table 4 we present similar data from the second patient. During the treatment with corticotropin

TABLE 3.—*Urinary Excretion of Amino Acids and Copper in Wilson's Disease: Influence of Treatment with Dimercaprol in Patient J. R.**

	Before Dimercaprol†			During Treatment with Dimercaprol (400 Mg./Day for 10 Days)†				
Blood amino nitrogen, gm. × 1000‡	0.04	0.42	0.041	0.06	...	0.042	...	0.051
Urine amino nitrogen, gm./24 hr.§	0.7	0.82	0.76	0.78	1.0	...	...	0.89
Urine total nitrogen, gm./24 hr.‖	22	20	21	17.6	17	...	...	17.3
Urine copper, γ/24 hr.¶	153	175	164	256	193	308	325	270

* Treatment with dimercaprol increases the urinary excretion of copper without affecting noticeably the excretion of amino nitrogen or the total nitrogen in urine or the amino nitrogen in blood.

† Columns indicate separate determinations, the average being given in the last column of each group.

‡ Bournigaut-Philibert method (normal, 0.05 gm./100 cc.).

§ Normal, up to 0.3 gm./24 hours.

‖ Method of Kjeldahl. Patient was given a diet containing 60 gm. protein.

¶ Method of Eden and Green (normal, up to 100 γ/24 hours).

TABLE 4.—*Urinary Excretion of Amino Acids and Copper in Wilson's Disease: Influence of Treatment with Corticotropin and Dimercaprol in Patient P. R.**

	Before Treatment†					Corticotropin (50 Mg./Day; Total, 250 Mg.)†			Dimercaprol (400 Mg./Day for 10 Days)†			
Blood amino nitrogen, gm. × 1000‡	0.05	0.06	....	....	0.055	0.081	0.094	0.087	....	0.052	....	0.052
Urine amino nitrogen, gm./24 hr.§	0.64	....	0.70	0.68	0.67	1.84	1.268	1.554	0.74	0.86	0.64	0.67
Urine total nitrogen, gm./24 hr.‖	15.4	16	17	....	16.4	19.50	33.62	26.51	20	18.3	....	19.1
Urine copper, γ/24 hr.¶	225	198	113	348	304 294 247	225	315	210 249	345	312	298	318

* Treatment with corticotropin increases the amino nitrogen in blood and the amino nitrogen and total nitrogen in urine. On the contrary, it does not increase the excretion of copper in the urine. Later treatment with dimercaprol increases the excretion of copper without changing perceptibly the urinary excretion of amino acid.

† Columns indicate separate determinations, the average being given in the last column of each group.

‡ Bournigaut-Philibert method (normal, 0.05 gm./100 cc.).

§ Normal, up to 0.3 gm./24 hours.

‖ Kjeldahl method. Patient was given a diet containing 60 gm. protein.

¶ Method of Eden and Green (normal, up to 100 γ/24 hours).

(ACTH) we observed a considerable increase in the amino nitrogen in the urine and in the total nitrogen in the urine (the diet of the patients had the same protein content, with more or less 60 gm. of protein a day), while the elimination of copper in the urine, on the other hand, shows no variation. We can therefore presume that protein catabolism induced by corticotropin mobilizes amino acids from tissues, while the blood amino acids and amino-aciduria increase in concentration, but, on the other hand, copper is excreted at the same rate as before (the patients showed no clinical improvement during this treatment with corticotropin). The dimercaprol administered some time later produced changes similar to those observed in the other brother, previously mentioned: increased elimination of copper in the urine (from an average of 247γ to an average of 318γ) during the treatment with dimercaprol, without showing any increase in the elimination of amino-acids during that time.

COMMENT

The data gathered from our cases give us sufficient evidence to support the statement that in hepatolenticular degeneration (at least in our cases) there is no affection of the renal function as is evident in the Fanconi syndrome. The increased ammoniopoiesis seems to depend upon the increased amino-aciduria. This phenomenon, however, precedes for several years the clinical onset of the disease, and, although the hepatic cirrhosis of Wilson's disease can be ascribed to the amino-aciduria, it is not the direct cause for the elimination of copper by the kidney, a finding so typical of this process; nor, in all likelihood, is it responsible for the retention of copper in the organism. In the Fanconi syndrome no neurologic lesions or alterations in the metabolism of copper are produced. On the other hand, according to our observations, the mobilization of copper and amino acids produced by the administration of dimercaprol and corticotropin, respectively, do not follow a parallel course.

Uzman and Hood [6] have found that in Wilson's disease, together with amino-aciduria, there is a highly increased urinary excretion of peptides. This peptiduria would probably overload the reabsorptive function of the tubule, which in the first place must cope with the reabsorption of these peptides and therefore cannot completely reabsorb the amino acids filtered by the glomerulus. The amino-aciduria would then be the result of a competitive reabsorption in the tubule, with predominance of the reabsorption of the peptides. According to Uzman and Hood[6] and, later, Brick,[25] the cause of Wilson's disease should be considered as a congenital familial alteration in which there is a lack of those enzymes necessary for the fragmentation or breaking down of the peptides to the state of amino acids. Protein breakdown, when such proteolytic enzymes are lacking, would be partially arrested in the phase of dicarboxilic oligopeptides, substances that deposit in tissues, especially in the liver, in the basal nuclei of the brain, and in the cornea. These peptides have a great affinity for copper; thus we can explain the increased deposition of this metal in the above-mentioned structures. The copper passed in the urine is bounded to these oligopeptides and not to the amino acids, this assertion being in accordance with Uzman,[26] who proved this by chromatographic studies after acid hydrolysis. At present we are studying the peptiduria in our patients, with the hope that it will give us some important data that will enable us to understand more clearly certain aspects of this disease.

SUMMARY

Results are presented of the study of a family in which two members have clinical and metabolic symptoms of Wilson's disease and a third has amino-aciduria as the sole symptom.

In the first two cases there was an evident increase in the formation of ammonia in the kidney; this finding together with the increase of the alkaline phosphatase (over which an overload with vitamin D had no influence) and the amino-aciduria shows a certain resemblance to the Toni-Fanconi syndrome, but the similarity between the latter syn-

drome and Wilson's disease is only in the above-mentioned finding and nothing more; it is dubious, in our opinion, that the amino-aciduria is a symptom of a nephropathia in a broad sense.

Corticotropin caused a heavy increase in the excretion of amino acids, with no change at all in the elimination of copper. Dimercaprol, on the other hand, increased the elimination of copper without affecting amino-aciduria. This lack of parallelism between the elimination of amino acids and copper after administration of corticotropin and dimercaprol is very interesting and demonstrates clearly, in our opinion, that copper is not eliminated by the kidney bound to amino acids, as some authors have considered. This is another point in support of Uzman's thesis that copper is excreted united to oligopeptids and not to amino acids.

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Treatment in Internal Medicine

TREATMENT OF PANCREATITIS

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ACUTE PANCREATITIS

THE TREATMENT of acute pancreatitis varies with its severity. In the milder attacks, presumably those due largely to edema of the organ, the need for control of pain often is the major and sometimes the sole indication for treatment. In the severer cases, in which necrosis of varying extent occurs, gastrointestinal and systemic symptoms, including disturbances of fluid and electrolyte balance; ileus with nausea, vomiting, and abdominal distention; shock; secondary infection, and food and vitamin deficiency states, develop and require management.

MEDICAL THERAPY

1. *Control of Pain.*—Drugs that relax the sphincter of Oddi, such as glyceryl trinitrate (nitroglycerin) given sublingually in doses of 0.6 mg. (1/100 grain) or papaverine hydrochloride in doses of 0.13 gm. (2 grains), theoretically should be tried first, because increased pressure within the pancreatic duct, which presumably follows spasm of the musculature of the duodenum and sphincter of Oddi induced by most opiates, presumably increases pancreatic damage. However, the danger of opiates appears to be more theoretical than real, and in practice pain relievers such as meperidine (Demerol) hydrochloride in doses of 100 mg. (1½ grains) and morphine sulfate in doses of 15 to 30 mg. (¼ to ½ grain) given hypodermically usually will be required to control pain. Methantheline (Banthine) has not been definitely helpful.

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2. *Maintenance of Normal Fluid and Electrolyte Balance.*—In the usual case of five to seven days' duration in which fluids cannot be taken orally in adequate amounts because of nausea and vomiting, sensible and insensible losses of water and chlorides must be estimated and replaced intravenously. Unless losses in vomitus or aspirations are large, the average daily requirements for fluids and chlorides are as given in the Table. The adequacy of these amounts often may be determined by urinary output, by the degree of hydration of the tongue and skin, and by the presence or absence of edema of the ankles. Determination of blood urea, plasma chloride, and carbon-dioxide-combining power of the plasma at appropriate intervals will give more accurate information.

Fluid and Chloride Requirements

Amount of Sweating	5% Dextrose in Water, Cc.	5% Dextrose + 0.9% Sodium Chloride in Water, Cc.
No sweating, no fever....	2000	500
High fever or moderate sweating	2000	1000
Severe sweating.....	2500	1000

Hypopotassemia and hypocalcemia may appear. When values for serum potassium are less than 3 mEq. per liter, particularly if hypochloremic alkalosis is refractory, 1 ampule of potassium chloride solution containing 1.49 gm. (20 mEq.) introduced into a liter of fluid is given intravenously. Total amounts given per day depend upon the degree of depletion and upon the behavior of values for serum potassium. Such therapy is given with great caution when urinary output is subnormal. When values for serum calcium fall below 8 mg. per 100 cc., calcium gluconate may be administered intravenously in doses of 10 to 20 cc. of a 10% solution, but the value of this has not been clearly demonstrated.

3. *Management of Ileus.*—Continuous gastric aspiration serves to empty the stomach and reduce vomiting. If distention persists, intubation of the small bowel may be needed to decompress the bowel.

4. *Shock.*—Transfusions of whole blood, human serum albumin, and plasma volume expanders such as Dextran may be used to restore blood volume. The danger of viral hepatitis precludes the use of plasma except under the most unusual conditions. It has been postulated that trypsin liberated during the acute destruction of the pancreas is responsible for toxicity and shock and that the beneficial effects of whole blood and plasma depend upon their antitrypsin content. However, recent studies have shown that the improvement in the condition of the patient after transfusion is well explained by the increase in blood volume. Maintenance of body warmth and elevating the foot of the bed are standard procedures. Corticotropin (ACTH) and cortisone have been advocated not only in the management of shock but also because of their anti-inflammatory effect; such therapy is difficult to evaluate in a disease as self-limited as acute pancreatitis is.

5. *Prevention of Secondary Inflammation, Abscess Formation, and Septicemia.*—The possibility of complications from infection must be suspected when moderately high fever appears, especially when fever of a septic type is present. The following measures serve to prevent or control these complications. A. Chlortetracycline (Aureomycin) or oxytetracycline (Terramycin) in doses of 500 to 750 mg. every six hours should be swallowed with milk; these antibiotics, however, should be used with caution in hospitals in which strains of Micrococcus pyogenes resistant to them are prevalent. B. If antibiotics cannot be taken orally, penicillin procaine in a dose of 1,000,000 units and dihydrostreptomycin in a dose of 1 to 2 gm. are given intramuscularly daily. C. If measures listed under B are ineffective, chlortetracycline or oxytetracycline in a dose of 500 mg. in 200 cc. of isotonic saline solution is given intravenously twice a day. The chance of damage to veins is reduced if the drug is given rapidly.

6. *Reduction of Pancreatic Secretion So That Abnormal Pressure Within the Pancreatic Ducts Will Not Rupture Them and Cause Progressive Damage to the Organ.*—That increased intraductal pressure occurs during acute pancreatitis has not been proved, but if present it may be reduced by a slow rate of pancreatic secretion. Theoretically this is accomplished by (*a*) avoidance of stimulation of the secretin mechanism by preventing entrance of acid gastric contents into the duodenum (continuous gastric aspiration) and (*b*) suppression of pancreatic secretion by the administration of drugs, such as atropine sulfate in doses of 0.6 mg. (1/100 grain) and ephedrine sulfate in doses of 25 mg. (⅜ grain) administered subcutaneously as often as every three hours or, when medication may be taken orally, methantheline in doses of 25 to 50 mg. at six-hour intervals.

7. *Parenteral Feeding.*—Dextrose given intravenously in the amounts mentioned in the Table may be supplemented with amino acids when the attack lasts longer than a few days. The salt content of the amino acid solution for intravenous use should be considered in determining the amount of sodium chloride given daily. Vitamin supplements may be added to the fluid to be given intravenously. As recovery takes place, and as soon as nausea and vomiting cease, fluid and food may be taken by mouth as tolerated.

Surgical Therapy

Acute pancreatitis usually does not require surgical intervention. However, surgical consultation always is advisable and certain procedures may be indicated. These include (1) block of paravertebral sympathetic nerves by injection of procaine or alcohol or both, resorted to when opiates fail to control pain, (2) drainage of peripancreatic abscess or inflammatory cyst (residues of pancreatic necrosis) and of pseudocystic collections of fluid in the lesser peritoneal cavities (such drainage usually may await recovery from the severe phase of the process or until the

condition of the patient is satisfactory for surgical intervention), and (3) exploration and appropriate surgical measures when clinical evidence is reasonably good for empyema or rupture of the gall bladder.

CHRONIC PANCREATITIS

Each attack of acute pancreatitis leaves residues of fibrosis, atrophy, and calcification. When destruction of the acinar and islet cells is extensive, diabetes and steatorrhea with creatorrhea appear. Treatment is threefold in purpose: (1) to prevent the recurring painful seizures and progression of the disease, (2) to control pancreatic insufficiency, and (3) to control intractable pain and other late sequelae. Both medical and surgical therapy must be utilized in the management of this difficult problem.

Medical Therapy

Medical measures for the prevention of recurring painful seizures and progression of the disease are limited in number and value. They are (1) ingestion of a bland diet, (2) avoidance of overeating, and (3) complete abstinence from alcohol.

Control of pancreatic insufficiency entails (1) control of the diabetes mellitus, by accepted methods, and (2) control of the excessive loss of fat, with its associated abdominal discomfort and the excessive loss of nitrogen (azotorrhea). Measures are two in number: a dietary program and the use of pancreatin. The greater the intake of fat and nitrogen the greater the absorption of these foodstuffs. Consequently, the ingestion of fat and nitrogen in amounts greater than those usually consumed by normal persons is the simplest method of combating the effects of excessive loss of these substances. Such an approach is satisfactory in respect to protein, but abnormally large amounts of fat in the diet may well increase the diarrhea and associated abdominal discomfort. In practice it is best to restrict the intake of fat, increase the ingestion of protein to permit increased absorption, and provide large amounts of carbohydrate to compensate for the calories lost by the restriction of fat intake. The resulting diet is of high caloric value (2,500 to 3,000 Cal.) with 120 gm. of protein, 50 to 70 gm. of fat, and up to 450 gm. of carbohydrate, depending upon the size of the individual person. The use of hydrolyzed protein is an unnecessary dietary adjunct. Concentrated triple-strength pancreatin as enteric-coated tablets (0.3 gm., or 5 grains, in each) is given in doses up to 5 gm. (15 tablets) with each meal. Pancreatin in such amounts will reduce losses of fat and nitrogen by approximately 50%, permit ingestion of larger amounts of fat, and reduce discomfort. Smaller doses may control abdominal discomfort without reducing greatly the fecal losses. In my experience sorethytan (20) monooleate (Tween 80) has not proved to be of value.

Other measures include the administration of vitamin supplements, such as 1 capsule of Multicebrin a day, especially when the patient is malnourished and the intake of food is restricted, and the administration of ferrous sulfate or ferrous gluconate by mouth and whole blood by transfusion if the patient is anemic. Other drugs, such as calcium gluconate, liver extract, and choline, seldom, if ever, are needed.

Surgical Therapy

After recovery from the acute attack the patient should undergo examination of the biliary and gastrointestinal tracts. Even if disease is not demonstrated on examination, abdominal exploration may be advised to exclude disease of the biliary tract and to provide opportunity for performance of one of the surgical measures currently advocated. The procedures vary according to the findings and conditions in the individual case.

Measures Currently Advocated in Cases in Which Recurrent Acute Attacks Are Separated by Periods of Relative Freedom from Symptoms.—1. Measures Directed Toward Correction of Disease of Biliary Tract: (*a*) If the gall bladder contains stones or is definitely diseased, cholecystectomy is performed. If the gall bladder is not diseased it is preserved.

(*b*) The common bile duct is explored routinely, with removal of stones if present. In those cases in which the pancreatitis is secondary to disease of the biliary tract, the removal of the disease of the biliary tract is highly effective in preventing future episodes of pancreatitis.

2. Measures Directed Toward Prevention of Recurring Attacks in Cases in Which Disease of Biliary Tract Is not Present or Has Been Removed: (*a*) The biliary tract is drained either by prolonged (6 to 18 months) drainage of the common bile duct with a T-tube or by some form of biliary-intestinal anastomosis, which preferably consists of joining the common bile duct to the duodenum (lateral choledochoduodenostomy) but which in exceptional cases consists of cholecystenterostomy if the gall bladder appears normal and the common bile duct is not particularly suitable for anastomosis; sphincterotomy of the transduodenal type may be performed, perhaps in association with prolonged drainage of the common bile duct with a T-tube, the long limb of which is passed through the sphincter of Oddi into the duodenum. These procedures are followed by reduction in severity and frequency of the painful seizures in about half the cases.

(*b*) Measures may be directed toward reduction of pancreatic secretion. These measures include vagotomy and partial gastric resection. The value of this approach apparently is not well enough substantiated to justify its employment.

(*c*) Measures are directed toward correcting conditions in the pancreas itself. These measures include removal of stones from the pancreatic duct (pancreaticolithotomy) (this does not include removal of calcified material from the parenchyma) and adequate internal or external drainage of any pancreatic cyst or peripancreatic abscess that may be present.

(*d*) Measures are directed toward such conditions as duodenal obstruction and secondary involvement of the stomach, small bowel, or colon.

Measures Currently Advocated in Advanced Cases in Which There Is Constant Pain with Exacerbations and Pancreatic Insufficiency.—1. Abdominal Exploration: If exploration has not been done previously, it should be done to determine the status of the biliary tract and the pancreas and to carry out previously mentioned procedures which, when indicated, are of possible value. If previous measures have been of temporary or of no value, then procedures designed specifically to control pain may be carried out.

2. Measures for Relief of Pain: These measures include (*a*) splanchnic block with procaine or alcohol; if relief is unsatisfactory, then (*b*) dorsal sympathectomy or splanchnicectomy, which usually should be bilateral, and (*c*) total pancreatectomy as a last resort.

METABOLIC FUNCTION OF THE PITUITARY GROWTH HORMONE

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RECENT progress in purifying hormone preparations of the adenohypophysis has greatly facilitated the tracing of the metabolic effects of the anterior lobe to specific actions of the individual pituitary principles. Among these, the growth hormone (GH), or somatotrophic hormone (STH), has lately been studied intensively. The findings have thrown some light on the means by which this hormone controls protein, carbohydrate, and fat metabolism without the mediation of any target gland.

PROTEIN

Two major metabolic changes have long been considered prominent features of the effect of GH on protein or nitrogen metabolism: (1) a decrease in the urinary excretion of nitrogen and (2) a fall in the concentration of nonprotein, mainly amino acid, nitrogen in the blood and tissues.

Decreased urinary excretion of nitrogen (especially in the form of urea) has been taken as evidence of an accumulation of nitrogenous material available for anabolic processes leading to cell growth and multiplication. The reduction in the nonprotein nitrogen level of the blood has also been interpreted to indicate enhanced protein synthesis.*

The nitrogen-retaining function of GH has been thought the primary cause of its most obvious effect: the increase in body size that represents true growth.† This view is supported by the observation that the carcasses of animals treated with pituitary growth preparations show a significantly higher content of protein than do the carcasses of control animals.‡ Conversely, animals from which the pituitary gland has been removed contain, on the average, less protein in their carcasses than do intact animals restricted to the same food intake.[189] These findings indicate that hypophysectomized animals are less able than controls to build up protein, although, in animals without the pituitary, the formation of new protein still proceeds at a significant rate.[291]

There is much evidence to show that the growth induced by the hormone is not limited to any specific tissue but occurs in all cell systems, such as muscle, skeleton, inner organs, and connective and granulation tissues.§ Moreover, it has been established that this growth can be maintained indefinitely under continued hormone treatment and unlimited food intake.‖ The growth is found in hypophysectomized, adrenalectomized, and thyroparathyroidectomized animals ¶; this suggests that the hormone acts more or less independently of any other pituitary principle.

The extent to which GH affects nitrogen metabolism depends not only on the dose of the hormone but also on the metabolic state of the animal. Especially important is the amount of nitrogenous metabolites available for protein synthesis. The retention of nitrogen was found to be increased in well-fed animals; it was even greater after animals had been given amino acids or corticotropin. In the fasting and in the adrenalectomized animal this hormonal effect was diminished or abolished.#

* References 159, 187, and 200.

† References 95, 159, 199, 204, 312, and 325.

‡ References 37, 84, 130, 188, 199, 203, 318, and 335.

§ References 22, 35, 80, 95, 131, 135, 136, 255, 283, 286, 296, 310, 311, and 340.

‖ References 97, 101, 130, and 296.

¶ References 92, 99, 115, 149, and 197.

References 29, 144, 271, 272, and 317.

The precise mechanism and the initial steps involved in the process of storing nitrogen and augmenting tissue protein are still obscure. The question, however, whether this effect comes from an enhanced formation of new protein or from an inhibition of protein breakdown can now be answered. Sufficient evidence has been accumulated to show that GH induces a shift of the balance between the anabolic and catabolic phases of protein metabolism in favor of increased synthesis.*

It has been suggested that GH exerts primarily an inhibitory effect on catabolic processes below the amino acid level, especially on urea formation. In fasting rats and in dogs, the breakdown of amino acids was found to be depressed during the period when nitrogen was stored under the influence of the hormone.† In nephrectomized rats formation of urea from injected protein hydrolysate was diminished after the animals had received crude or purified GH preparations.‡

From all of these data it has been concluded that the hormone-induced growth is the resultant of two principal effects: a reduced rate of amino acid catabolism and an increased rate of protein synthesis.[20]

The nature of the protein synthesized under the control of GH has not yet been elucidated. The significant increase in the plasma volume of the blood is due to an increase in the amount of total protein; the high molecular fractions, like globulin and fibrinogen, are especially augmented.[60] In the tissues the excess protein deposited appears in a labile, functionally ineffective form §; this protein is probably transformed secondarily under the influence of the thyroid into specific, physiologically active, tissue components.[340]

There is a close relationship between the effect of GH on nitrogen anabolism and the function of the islet apparatus. Some investigators || found that after GH administration the protein synthesis was diminished or abolished when insulin was lacking; several others ¶ reported that GH decreased the urinary excretion of nitrogen and reduced the blood amino nitrogen to a similar extent in normal and diabetic animals. According to recent evidence, GH in the depancreatized hypophysectomized cat causes no retention of nitrogen; there is partial retention of nitrogen in the depancreatized animal if it is given a certain amount of insulin. By increasing the doses of insulin, retention of nitrogen can be restored to normal. It has been further noted that at a constant level of insulin in the blood, nitrogen retention varies with the GH dose.[231]

These observations support the view that insulin is essential to the anabolic effect of the GH on protein; they indicate further that, normally, an increased secretion of insulin occurs in response to the GH effect.#

It is known that insulin itself promotes growth,[279] that it induces a retention of nitrogen, a fall of the level of amino acids in the blood, and a reduction in urea formation.* These findings suggest that insulin promotes protein synthesis in a way similar to that of GH.†

Since the action of GH on nitrogen has been found to be, to some extent, independent of the pancreatic function, it has been considered unlikely that the effect of the GH on protein anabolism is mediated by stimulation of the pancreas.‡ Growth may rather be assumed to result from a synergistic action of GH and insulin § supported by a number of other endocrine principles, such as the thyroid hormone || and the androgens,¶ in addition to nutritional factors.

* References 20, 111, 149, 212, 271, 272, 275 and 296.

† References 20, 21, and 149.

‡ References 271 and 274.

§ References 38, 125, and 132.

|| References 80, 212, 234, and 326.

¶ References 23, 26, 28, 197, and 230.

References 149, 228, and 231.

* References 43, 64, 108, 145, 164, 184, 211, 233, and 274.

† References 149 and 105.

‡ References 2, 26, 30, 149, 166, and 231.

§ References 108 and 271.

|| References 100, 149, 186, and 259.

¶ References 18, 177, 181, 324, and 330.

CARBOHYDRATE

The effect of anterior pituitary extracts on carbohydrate metabolism was early associated with their growth-promoting qualities.[152] From the beginning, however, it was recognized that the various changes in carbohydrate metabolism seen after administration of hypophyseal extracts could not be accounted for by the action of one single hormone. When hypophyseal extracts were found to exert a significant effect on sugar metabolism even after the influence of one major pituitary factor, corticotropin, had been eliminated, by removing the adrenal glands, investigations centered mainly around the growth-promoting principle.#

Recent studies with purified GH preparations have removed some doubt about the diabetogenic potency of the pituitary growth factor. Although a homogeneous protein with invariable physicochemical properties has not yet been obtained, it has been shown by methods of partial inactivation that the same structures within the protein molecule account for both growth stimulation and the inducing or intensifying of diabetes.* These investigations have also greatly increased understanding of the mechanism by which the hormone produces the various changes in carbohydrate metabolism: the glycostatic, glycotropic, anti-insulin, diabetauxetic, and diabetogenic effects.

In the normal adult animal (dog, cat, and ferret) and in the chick embryo, GH, in proper doses over an extended period, has produced the typical signs of diabetes: sustained rise in blood sugar level, glycosuria, polyuria, polydipsia and ketonuria.† Existing diabetes has been significantly aggravated in animals (dogs, cats, rats, batrachians) partially or totally depancreatized or treated with alloxan.‡

In contrast to the hyperglycemic effect of protracted administration of GH, a single injection is followed by a fall in the level of blood glucose when the hormone is given to a normal fasting rat or hypophysectomized dog.§ (It does not have this effect on a normal dog.[77]) In the adrenalectomized rat the hypoglycemia may be severe and even fatal. This effect is not seen in alloxan-diabetic rats; these respond with a rise in the concentration of blood glucose. The hypoglycemic phase has therefore been attributed to an increased output of insulin in response to the GH action.

That GH is a potent stimulant for the elaboration and secretion of insulin by the β-cells has been suggested by much physiological and histological data gained from acute and prolonged studies with GH or anterior pituitary extracts of high activity in promoting growth. Early in the treatment, the islet cells proliferate and hypertrophy.|| Reflecting different phases of secretory activity, the insulin content increases or decreases, according to the species of the animal.¶ Later, if stimulation is protracted, there are degenerative changes and a reduced output of insulin. These changes indicate a state of exhaustion from continued strain beyond the functional capacity of the β-cells.#

The nature of this "pancreotropic" function of GH has not been clearly defined. It is assumed that the pancreatic β-cells react to the hormone-induced hyperglycemia by increasing the output of insulin.* We know that the islet cells respond very promptly to fluctuations in the glucose level of the blood. It has been shown, moreover, that a persistent rise in the blood sugar concentration can severely damage the islet cells.[83] Apparently, however at least one additional regulatory mechanism must be involved in producing hypoglycemia after a single injection of GH.

References 24, 148, 155, 216, 273, 293, and 331.

* References 60, 146, 158, 259, 261, and 339.

† References 56, 57, 60, 62, 157, 160, 229, and 231.

‡ References 26, 56, 61, 113, 123, 156, 212, 223, 228, 230, 231, and 271.

§ References 214, 224, 228, and 230.

|| References 138, 140, 245, 264, and 265. Abrams and co-workers [2] found only slight and inconsistent cytological changes of the β-cells in force-fed rats treated with GH.

¶ References 33, 60, 107, 221, and 334.

References 34, 60, 62, 230, 231, 265, and 335.

* References 4 and 329.

The drop in blood sugar after administration of hormone has been observed before any elevation of the glucose level.[230] This phenomenon can hardly be explained by a pancreatic response to a primary hyperglycemia. In experiments on isolated rat pancreas, a decrease rather than an increase in the release of insulin was noted when GH was added to the perfusate.† The significance of these findings is difficult to evaluate in the light of all the other experimental results.[30]

Contrary to the results of experiments on alloxan-diabetic rats,[23] temporary hypoglycemia after intravenous injection of GH has been observed in the acutely depancreatized dog, an effect which, at least in part, would have to be explained by an extrapancreatic mechanism. After repeated GH injections, the reactive hypoglycemia gradually diminishes and, if treatment is continued, turns into the hyperglycemic effect.[28] This change, in turn, is followed by a true diabetic state as the functional reserve of the islet apparatus becomes exhausted.

Unlike normal dogs and cats, hypophysectomized animals do not exhibit the characteristic signs of diabetes after being treated with GH. In them the most conspicuous manifestation of the hormone action is its anti-insulin effect: The GH diminishes and even abolishes the exaggerated hypoglycemic response to exogenous insulin, the hypersensitivity to insulin following hypophysectomy.‡ When those animals are given GH over a prolonged period they develop a resistance even to large doses of insulin; this resistance may surpass that of normal control animals.[72]

The diminished response to insulin, induced by GH, has been noted not only in hypophysectomized and adrenalectomized-hypophysectomized animals but also in normal and diabetic animals[228]; it has been seen, too, in hepatectomized rabbits,[145] in eviscerated rats,[25] and in the isolated diaphragm of the hypophysectomized rat.[300] These findings suggest an extrahepatic mechanism for the insulin resistance.

In addition to the anti-insulin effect, elevated postabsorptive sugar levels of the blood and diminished glucose tolerance have been observed after administration of GH to hypophysectomized as well as to adrenalectomized animals.§ Such a response was not found in the adrenalectomized cat.[208]

Although neither glycosuria nor ketonuria appears in animals after the pituitary is removed, the increased resistance to exogenous insulin, the impaired tolerance to glucose, and the elevated blood sugar level are thought to reflect a diabetic state.‖ It has been suggested that these metabolic alterations in hypophysectomized animals do not differ in principle from the diabetogenic effect of GH in normal animals and its aggravation of diabetes in animals already diabetic; rather, it is thought, all these phenomena are manifestations of one basic function of the GH, the inhibition of the peripheral utilization of carbohydrate.¶

It must further be assumed that GH given to the normal animal provokes secretion of additional insulin, which restores the metabolic equilibrium. As a result, the decreased utilization of carbohydrate becomes apparent only when the islets no longer are capable of adjusting to the alteration in carbohydrate metabolism by increasing insulin output.#

The degree of those metabolic changes, from slight elevation of the glucose-tolerance curve and increase in insulin resistance to severe diabetes, is obviously determined by the dose of the hormone and the duration of its administration. It also depends, however, on the functional capacity of the islet apparatus, which again varies with the species of the animal, its nutritional state, and its age, the younger animal exhibiting much greater resistance to the diabetogenic action of the GH than the older animal.* Accordingly, any reduction of pancreatic function by

† References 4 and 5.

‡ References 23, 72, 79, and 230.

§ References 72, 208, and 230.

‖ References 72, 230, and 271.

¶ References 72, 230, and 271.

References 61, 72, 230, and 231.

* References 72, 90, 230, 333, 334, 337, and 339.

chemical or surgical means greatly enhances the effect of GH on carbohydrate metabolism. After partial pancreatectomy, the sensitivity to the hormonal action was found to be inversely proportionate to the decrease in the islet tissue.[156]

Another significant effect of GH action on carbohydrate, the glycostatic phenomenon, has been considered to indicate an impairment of carbohydrate utilization. The rapid disappearance of glycogen stores in hypophysectomized fasted animals and the concomitant rise of the respiratory quotient have been shown to result from an abnormally high rate of carbohydrate oxidation.† Crude anterior pituitary extracts have been known to counteract this rapid depletion of the glycogen depots in fasting hypophysectomized animals; they do so, apparently, by reducing the glucose requirement of the tissues, and thus they maintain the normal muscle glycogen stores even though the blood glucose and liver glycogen are at subnormal levels.

The glycogen-sparing effect of crude extracts was demonstrable in the hypophysectomized-adrenalectomized rat; evidently, this effect is brought about independently of the function of the adrenal cortex.[80] Recent experiments with highly purified preparations of GH have furnished additional evidence to show that in fasted hypophysectomized animals, the hormone restores the depleted glycogen depots and lowers the respiratory quotient by diminishing the net utilization of carbohydrate.‡ The glycostatic phenomenon exhibited in the hypophysectomized animal has also been observed in normal fasting and glucose-fed rats, which, under the influence of the GH, show increased deposition of glycogen in the muscles.§ The glycogen content of the liver was found increased by some investigators; others noted a depletion of the hepatic glycogen.[214] The inhibition of carbohydrate utilization by the hormone has furthermore been illustrated by in vitro experiments on the uptake of glucose by the rat diaphragm; the rate of the glucose uptake is increased after previous removal of the pituitary and reduced to normal when GH is given to the hypophysectomized animals.‖

Whether or not the inhibition of sugar utilization is the only mechanism responsible for the various changes in carbohydrate metabolism is still an open question. Stimulation of the pancreatic *a*-cells and the resulting increased secretion of a hyperglycemic-glycogenolytic principle (glucagon) have been considered a possible factor in producing the anti-insulin effect of GH.¶ There is very little evidence, at present, to support this view.

The significance of gluconeogenesis as a contributing factor in the GH effect on carbohydrate metabolism is difficult to evaluate on the basis of the available experimental data. In the hypophysectomized fasting animal, especially the dog, a marked reduction in the formation of glycogen is observed in addition to increased utilization of glucose.[30] These changes are abolished by administration of anterior pituitary extract. It appears, however, that the maintenance of carbohydrate stores by glyconeogenesis is primarily the result of corticotropin action and that GH is mainly concerned with the inhibition of carbohydrate utilization.#

Experiments on the force-fed rat and cat show that the diabetogenic effect of both hormones in combination surpasses that of each individually *; in the hypophysectomized dog, however, the cortical hormones are not found to enhance the effect of growth hormone but to modify it so as to restore a normal level of carbohydrate metabolism.[75]

FAT

Experimental work on the influence of GH on lipid metabolism has been proceeding for some time independently of the studies

† References 30, 77, 128, 129, 268, 269, and 276.

‡ References 91, 166, 167, 271, 276, and 327.

§ References 166 and 230.

‖ References 247, 248, and 299.

¶ References 46, 226, and 339.

References 30, 147, and 268.

* References 90, 258, and 260.

on the hormonal effect on protein and carbohydrate.

A relationship between the anterior pituitary and fat catabolism was established in early experiments.† Not until the investigations of Best and co-workers, however, was there provided a surer basis for understanding the alterations of lipid metabolism under the endocrine influence. These and later investigators ‡ showed that in fasting mice and rats certain ketogenic anterior pituitary extracts caused a rapid and intensive deposition of fat in the liver and, to a lesser extent, in the kidneys.

Experiments with deuterium § showed that these lipids, which were later found to be triglycerides,[58] accumulate at the expense of preexisting depot fat. In contrast to the increased fat content in liver and kidneys, the total amount of body fat diminished markedly when animals were treated with those hypophyseal extracts.‖ Since considerably more fatty acids disappeared from the depots than were found in liver and kidneys, it was concluded that the mobilization of stored lipids is accompanied by enhanced utilization of fat. This view was consistent with the findings of increased formation of ketone bodies, depressed respiratory quotient, and raised oxygen consumption.¶ It was also noticed that there is no rigid quantitative relationship between the amount of fat deposited in the liver and the level of ketone bodies in the blood, but that the rates of fat mobilization and the oxidative breakdown of fatty acids vary independently with the species and sex of the animals.#

Further studies with partially purified anterior pituitary preparations yielded evidence that the hypophyseal factor which promotes fat catabolism is closely related to those which stimulate protein anabolism and inhibit carbohydrate utilization.* Young, in particular, emphasized the intimate association between the functions of these pituitary extracts in promoting growth, intensifying diabetes, and catabolizing fat. He considered the over-all effect to be the result of a depressed oxidation of carbohydrate and protein combined with an increased rate of fat combustion.

Recent experimental work with highly purified GH preparations has confirmed the earlier findings of the hormonal effect on lipid catabolism. It has been shown that GH (*a*) reduces the fat content of the carcass †; (*b*) elevates the lipid level of the blood serum, chiefly the level of neutral fat ‡; (*c*) causes a rapid accumulation of these triglycerides in the hepatic cells, especially in the fasting normal and hypophysectomized animal §; (*d*) stimulates formation of ketone bodies in the normal and diabetic animal ‖ and in the fasting man ¶; (*e*) depresses the respiratory quotient as long as it maintains the increased rate of growth.#

These findings strongly suggest that GH induces accelerated mobilization and oxidation of depot fat. Furthermore, present evidence indicates that the two processes are not completely coordinated in extent and speed of reaction. Rather it appears that in the early stage of hormone action fat mobilization predominates, with the result that lipids accumulate in blood, liver, and, to a lesser degree, in other organs; at a later stage (usually after 24 hours), a decrease in the fat content of blood and liver reflects a restored equilibrium between the mobilization and further disposition of this material.*

In contrast to the depletion of the fat stores of animals treated with GH, there is

† References 7, 51, 151, 218, and 253.

‡ References 31, 32, 52, 53, 59, 112, 126, and 219.

§ References 16 and 302.

‖ References 84, 188, 243, and 318.

¶ References 12, 31, 58, 59, 66, 232, 243, 313, and 314.

References 31 and 243.

* References 95, 127, 188, 294, and 335.

† References 130, 181, 203, and 322.

‡ References 60, 71, and 133.

§ References 60, 71, 133, 204, 210, 214, 236, 251, 290, 308, and 320. Levin and Farber (References 194 and 196) found little or no increase in liver fat in mice treated with purified GH preparations.

‖ References 28-30, 45, 60, 134, 214, and 317.

¶ References 44, 49, and 63.

References 130 and 230.

* References 133 and 204.

a relatively high content of lipids in the carcasses of hypophysectomized animals. This fat-sparing effect of hypophysectomy, which is exhibited by animals on a suboptimal diet as well as by those that are force-fed, indicates a reduced ability to mobilize lipids from the depots.† GH has been found to lower the fat content in hypophysectomized animals to the same extent as in normal animals, in which the total lipids are reduced in inverse proportion to the increase in protein and water.[203]

There is still some uncertainty whether or not GH is the principal factor controlling lipid metabolism.‡ Earlier findings that adrenalectomy abolishes the accumulation of fat in the liver after administration of pituitary extracts promoting growth § have drawn the attention of many investigators to the adrenal cortex.[196] Although this gland is no doubt important in the hormonal control of fat metabolism, no evidence has yet been presented to show any direct stimulatory effect of cortical hormones on fat mobilization.‖

Corticotropin and the cortical oxysteroids, which counteract the GH influence on protein ¶ and enhance its effect on carbohydrate,# on the one hand stimulate formation of ketone bodies *; on the other hand, they inhibit ketosis induced by fasting,[171] fat-feeding,[172] or cold stress.[282] The hormones promote the deposition of lipids (glycerides) in the liver †; however, they do not appear to accelerate mobilization of neutral fat from the stores ‡ but, in the acute experiment, exert a fat-sparing effect [183] and even tend to augment the content of fat in the carcass,§ especially in the hypophysectomized rat.‖ Moreover, they reestablish deposition of fat ¶ which has decreased after adrenalectomy.# Therefore, the excess ketone bodies in the blood and fatty acids in the liver after administration of corticotropin or cortical hormones must be assumed to originate from sources other than the depot fat, possibly, at least partly, from the ketogenic and glucogenic intermediates of protein catabolism.[29] It is not hard to explain why the effect on liver fat of the combined action of corticotropin and GH, whether in crude extracts or purified, surpasses that of either one alone.* The reports that GH does not induce accumulation of lipids in the liver after the adrenals have been removed have not been confirmed by recent investigations. It has been found that the fatty infiltration of the hepatic cells produced by purified GH is not totally dependent on the presence of the adrenals,† just as there is an increase in ketone bodies in the blood after GH administration in the absence of the cortex,‡ although to a much lesser degree than in the intact animal.[317] It has been suggested, therefore, that the results in normal and adrenalectomized animals differ only quantitatively and that the cortex does not mediate but rather permits,[163] facilitates,[309] or conditions [287] the response to GH, as a result of its general physiological function.

Some of the other pituitary hormones § and a separate new principle [251] have been claimed to influence mobilization of stored

† References 116, 189, 191, 277, and 278.

‡ References 193, 195, 251, and 322.

§ References 112 and 219.

‖ References 162, 195, 251, and 308. The findings of an increase in fat catabolism after administration of corticotropin in phloridzinized rats (Segaloff and Many) and in diabetic subjects (Kinsell and co-workers, References 173 and 175) might be interpreted as resulting from simultaneously increased pituitary secretion of GH.

¶ References 27, 28, 89, 149, 182, 197, 250, and 287.

References 23, 27, 28, 78, and 165.

* References 29, 206, and 284.

† References 14, 165, 204, 236, and 290.

‡ References 165, 166, 182, 193, 230, and 251.

§ References 170, 178, and 295.

‖ Reference 203. Welt and Wilhelmi [322] found reduced content of fat in the carcass both after adrenalectomy and after corticotropin treatment.

¶ References 281 and 323.

Reference 328. Lipsett and Moore [206] did not find any difference in the fat content of the livers of fasted normal and adrenalectomized mice.

* References 194 and 251.

† References 210 and 309.

‡ References 29 and 308.

§ References 9, 168, and 262.

fat; however, the significance of those factors in the intermediary lipid metabolism has not been sufficiently established to permit a definite role in fat catabolism to be attributed to them. The bulk of the experimental work tends to show that the main hypophyseal principle controlling fat mobilization and utilization (lipoidrin [258]; adipokinin ||) is identical with GH.

CONCLUSIONS

In summarizing the principal changes in the turnover of protein, carbohydrate, and lipids under the influence of GH, the over-all effect of GH on intermediary metabolism may be considered the result of three basic alterations: (1) retention of nitrogen, associated with increased synthesis of tissue protein; (2) impairment of the utilization of carbohydrate, perhaps to a minor extent, enhanced gluconeogenesis; (3) increased mobilization of neutral fat from the depots with consecutive acceleration of fatty acid breakdown.

It appears highly improbable that these metabolic effects should result from simultaneous independent hormone action on protein, carbohydrate, and fat. Therefore, the question arises whether or not it is possible to trace all the various metabolic changes to one single point of attack.

It has been suggested [314] that a primary inhibition of protein mobilization and utilization under the influence of GH may compel the organism to utilize the depot fat for its energy requirements. Increased fat mobilization would be the result. However, there are several factors that can hardly be interpreted in favor of this theory, even if we substitute promotion of protein synthesis for inhibition of protein catabolism: these are the changes in carbohydrate metabolism and the magnitude and early onset of fat mobilization.¶

Changes in carbohydrate metabolism have also been considered the primary effect of GH action.[214] However, such a hypothesis involves even greater difficulties in elucidating the complex nature of the hormonal influence on the entire intermediary metabolism.

On the other hand, evidence, as presented above, appears entirely consistent with the concept of a primary hormone action on lipid metabolism with secondary changes in the turnover of nitrogen compounds and carbohydrates. A number of investigators have considered enhanced utilization of fat as a possible mechanism for the protein-anabolic effect of crude anterior pituitary extracts and purified GH preparations.# According to this view, one would have to assume the following sequence of events: mobilization of depot fat and its transportation to the liver and other organs is followed by excessive oxidative breakdown of fatty acids, particularly in the hepatic cells, but also in extrahepatic tissues,* leading to an accumulation of 2-carbon fragments within the common metabolic pool. These short chain units are utilized in several ways:

A. One portion undergoes immediate oxidation through the citric acid cycle. This process yields the energy required for synthetic processes, e. g., peptide bonds.†

B. Another portion can be used as building material for protein assimilation.[198] It has been amply demonstrated that amino acids are synthesized via the tricarboxylic acid cycle from short chain compounds of the acetate type.‡ One may then assume that the two-carbon units in combination with intermediate products of carbohydrate metabolism § form amino acids by taking over nitrogen from fragments of normal protein degradation, mainly by transamination. An increase in aspartic-glutamic transaminase was found in the liver under GH influence in hypophysectomized or underfed normal rats.[21]

Accumulation of intracellular amino acids at the expense of plasma nonprotein nitrogen

|| The term adipokinin was proposed by De Witt Stetten Jr. (Weil and Stetten [319]).

¶ References 53, 133, 308, and 320.

References 133, 188, 209, 308, and 341.

* References 124 and 142.

† References 150, 204, and 321.

‡ References 3, 6, 8, 39, 122, 239, 252, and 266.

§ References 122, 150, 185, and 301.

has been shown to facilitate formation of tissue protein [65] and thus to lead to true body growth. Insofar as nitrogen is incorporated into excess protein, it is prevented from being transformed into urea and excreted by the kidneys. Such a mechanism of GH action may be considered the physiological basis of the phenomenon of nitrogen retention or positive nitrogen balance as well as the basis of the reduction in the nonprotein nitrogen compounds in the blood; it would explain a number of other observation, e. g., the high G/N ratios in phloridzinized animals after corticotropin [284] and the prevention by GH of metabolic changes induced by stress or by the action of corticotropin.[285]

The importance of the carbohydrates for protein anabolism is well established. Nitrogen retention has been noted regularly after addition of carbohydrate to adequate or submaintenance diets.[237] Increase in the concentration of sugar in the blood by glucose injection has been found to decrease the rate of urea formation in the nephrectomized rat, i. e., to inhibit amino acid catabolism, both at its normal rate and when enhanced either by addition of exogenous amino acids or induced endogenously by added corticotropin and oxycorticosteroids.‖ Furthermore, after glucose administration there is a decrease in the content of amino acids in the blood in normal and diabetic subjects and in hepatectomized dogs.¶ In vitro, the amino acid uptake by liver and diaphragm of rats is accelerated when glucose is added to the medium.[184] The mechanism by which glucose increases protein anabolism is not clearly understood.[237] However, synthesis of the nonessential amino acids from carbohydrate metabolites has been shown repeatedly.[301] Therefore, it may be assumed that the intermediary compounds of glycolysis are used as building elements for the formation of amino acids, besides being oxidized to yield energy.

The anabolic effect of glucose on nitrogen metabolism is diminished or abolished by removing the pancreas [43] and intensified by adding insulin.# These results show that the utilization of carbohydrate for protein synthesis depends both on the sugar level of the blood and on adequate supply of insulin which promotes the glycolytic reaction series from blood glucose to the components of the Krebs cycle by accelerating the rate of hexosephosphate formation. With this primary action, the pancreatic hormone appears to achieve its nitrogen-assimilating and protein-sparing effect.

A conclusion, then, is that GH and insulin jointly promote protein anabolism by supplementing each other in providing the building material and energy required for the synthesis of the carbon skeleton of the nonessential amino acids.

C. A third major portion of the two-carbon fragments of β-oxidation is transformed mainly in the liver by random condensation to ketone bodies,[133] which are taken up by the bloodstream and carried to all tissues. Enhanced utilization of these excess products of the breakdown of fatty acids, through the citric acid cycle, appears to inhibit the simultaneous oxidative catabolism of glucose,* just as excess carbohydrate intermediates decrease the extent of fatty acid oxidation in the competitive process of terminal breakdown by an enzymatic mechanism of limited capacity.† This phase of the adipokinetic action of GH may account for the ketogenic, glucose-tolerance-depressing, glycostatic, and anti-insulin phenomena which characterize the GH effect in the hypophysectomized animal.

Besides the glycerin component of the neutral fats, a variable portion of fatty acids is transformed into glucose through the Krebs cycle.‡ Therefore some formation of new carbohydrate will result from the action of GH and enhance its effect on carbo-

‖ References 87 and 88.

¶ References 104 and 238.

References 108, 145, 164, 211, and 233.

* References 85, 94, 121, 217, 240-242, 249, and 315.

† References 117 and 118. The findings of Recant [257] which suggest a block between hexosephosphate and pyruvate are at variance with these conclusions.

‡ References 1, 42, 120, 303, and 304.

hydrate metabolism. However, the stronger gluconeogenic principle of the anterior hypophysis appears to be corticotropin, which, via the cortex, furthers the synthesis of carbohydrate from intermediates of protein catabolism. The intense stimulation of the islet cells in the rat by corticotropin [2] illustrates the high glucogenic potency of this hormone. GH and corticotropin may be considered the chief agents of the powerful diabetogenic effect of anterior pituitary extracts.§

The foregoing evidence suggests the pivotal role of the pancreas in controlling these metabolic processes. The glycolytic components of the common metabolic pool which are prevented by the action of GH from being oxidized or stored as lipids are available for protein anabolism. Together with the fragments of excessive lipolysis they are used for the synthesis of amino acids and polypeptides. The predominant effect of GH will be retention of nitrogen and growth, as long as the islet cells discharge sufficient hormone to secure an adequate supply of building material from glucose. If the output of insulin is insufficient, growth will be retarded or abolished and a diabetic tendency, but usually not frank diabetes, will appear. If, however, there is a simultaneous increase of corticotropin in the blood, the enhanced formation of carbohydrate precursors from protein and an intensified inhibition of glucose utilization will reverse the trend of the glycolytic process normally maintained by insulin. A true diabetic state will result whether the corticotropin is increased by simultaneous administration with GH or endogenously in hypophyseal response to the augmentation of plasma and tissue protein.

It has been suggested [90] that in certain animal species (e. g., the adult dog and cat) the cortical activity may be higher than in others (e. g., the rat); such variations would explain certain differences in the extent of the diabetogenic effect of GH. It has further been shown [90] that when animals are overfed hyperglycemia and glycosuria develop faster under GH treatment, apparently because of the additional strain on the islet apparatus and the resulting decrease in its functional capacity. On the other hand, in growing animals, such as puppies and kittens, diabetes usually cannot be produced by GH,‖ because of the ample functional reserves of the pancreas and the greater demand for protein. Any additional outlets for the surplus short-chain elements which accumulate under the influence of GH will lessen the glycotropic tendency and reduce the excessive requirement for insulin. This is seen in the pregnant or lactating animal, in which the growing fetus or the milk-producing mammæ absorb enough excess material to prevent the diabetogenic response to GH.¶ Consistent with these data are the findings that GH given to pregnant rats produces an increase in the birthweight of the offspring [93] and the observation that in the cow GH is a highly active galactopoietic agent.#

D. A fourth, probably smaller, portion of the two-carbon fragments of the breakdown of fatty acids is used for the synthesis of various specific metabolites, such as cholesterol.* A slight but significant increase in the cholesterol content of the blood in animals treated with GH has lately been reported.[225] These findings fit neatly into the over-all picture of the metabolic changes initiated by the fat-mobilizing action of the GH.

PHYSIOLOGICAL EFFECTS

Finally, the concept of a primary adipokinetic function of the GH promises to clarify the role which this hormone plays in controlling the intermediary metabolism under physiological conditions. GH produces precisely the changes characterizing the metabolic pattern during starvation, insofar as it inhibits the utilization of carbohydrate and of nitrogenous compounds for the generation of energy, by supplying the common metabolic pool with material from the fat

§ References 90 and 258.

‖ References 333, 334, and 337.

¶ References 90, 332, and 336.

References 69 and 146.

* References 41 and 110.

depots.† There is as yet no experimental evidence to show that GH is secreted in increased quantity by the pituitary during prolonged fasting. However, such a hypophyseal adaptation mechanism has been considered probable.‡ Some support for this view may be derived from the observation that in mice the fasting ketonemia is lowered after hypophysectomy but not after adrenalectomy.[11]

Obviously, GH is not the only pituitary principle controlling metabolism during starvation. The cortical steroids are thought more important than GH in keeping fasting hypophysectomized dogs temporarily alive, since these hormones provide the vital carbohydrates from nitrogenous material.[77] Yet a fatal protein deficiency would sooner or later ensue if GH did not simultaneously suppress the oxidation of carbohydrate and counterbalance the accelerated catabolism of protein. By doing so, GH replenishes the content of essential metabolites from expendable depot fat until this source is exhausted.[130]

On the other hand, under favorable nutritional conditions the adipokinetic action of GH balances the lipogenic effect exerted by excess exogenous metabolites piling up in the common metabolic pool. The hormone, by forcing these intermediary short-chain elements into the channels of nitrogen anabolism, enhances the assimilation of protein with the aid of insulin and other essential hormonal factors. In this way, it promotes true body growth.

SUMMARY

Recent experimental work on the influence of GH on the intermediary metabolism is reviewed. An attempt is made to trace the many different metabolic changes induced by the hormone to three fundamental processes: inhibition of the formation of urea from amino acids, repression of the oxidative breakdown of carbohydrate, and acceleration of the mobilization of fat from the depots. Mobilizing labile, stored lipids for various metabolic processes is considered the primary function of GH (adipokinin). Some pertinent experimental data are interpreted in the light of this theory.

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† References 133, 308, and 339.

‡ References 133, 308, and 339.

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News and Comment

ANNOUNCEMENTS

Robert Roesler de Villiers Foundation.—The Robert Roesler de Villiers Foundation, established specifically for the purpose of encouraging research directed at finding a means for a preventive measure, control, or cure of leukemia, will award grants-in-aid in support of research on leukemia. Qualified investigators are encouraged to apply to the Robert Roesler de Villiers Foundation, Inc., 1172 Park Ave., New York 28. The closing date for applications is Aug. 1, 1955. Grants-in-aid of not more than $1,000 each will be made beginning Sept. 1, 1955, as recommended by the selection committee.

Books

Heart Disease and Industry: With Particular Reference to Workmen's Compensation Cases. By Meyer Texon, M.D. Price, $7.50. Pp. 324. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1954.

As Dr. Levine points out in his foreword, there has been no comprehensive clinical treatise on the appraisal of cardiac disability as an industrial accident. Dr. Texon in the detailed presentation of 100 case reports analyzes every conceivable angle of the problem, which is stated in systematic fashion in a preliminary section. Aside from making intensely interesting reading, the book serves as a sort of "course" for the doctors who may be called on to render an opinion on the medicolegal aspects of trauma in relation to heart disease. Dr. Texon has made a most valuable contribution with his meticulous studies and the penetrating and skillful way in which he summarized his conclusions in each case.

Endemic Goiter: The Adaptation of Man to Iodine Deficiency. By John B. Stanbury, M.D., and associates. Price, $4.00. Pp. 206. Harvard University Press, Cambridge 38, Mass., 1954.

A real contribution to the better understanding of the physiology of the thyroid gland has been made by the authors, who studied thyroid function in 138 persons with goiters living in the province of Mendoza, Argentina. Goiter is endemic in this west central section of Argentina, where the food and water ingested by the native population is deficient in iodine. Most of the goiters encountered were nodular goiters, and most of the persons included in the study were euthyroid. Various aspects of thyroid function were studied as measured by the percent of a tracer dose of I^{131} collected by the gland and excreted into the urine, the serum protein-bound-iodine, and the amount of inorganic iodide excreted in the urine. Many interesting, important, and provocative observations were made in these patients by the authors, and they are set forth in the book in clear terms and a readable style. The data collected are analyzed mathematically, and the text is liberally illustrated with helpful graphs and tables. The reader is aided in his understanding of the facts given in each chapter by a few paragraphs at the end of the chapter summarizing the material presented and emphasizing the salient points. This opportunity of studying with modern methods a large group of persons with endemic goiters is unique, and the authors have reported their findings well. The book is not long (206 pages) and is worthwhile reading for any person interested in physiology of the thyroid.

Ulcerative Colitis and Its Surgical Treatment. By Bryan N. Brooke, M.D. Price, not given. Pp. 147, with 89 figures and 7 tables. E. & S. Livingstone, Ltd., 16 and 17 Teviot Place, Edinburgh 1; Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1954.

This profusely illustrated and handsomely made book is written by a surgeon from the surgical point of view, although the role of a team in the management of ulcerative colitis

is set forth clearly. Much emphasis is given to the differentiation of ulcerative colitis from ileocolitis and from proctosigmoiditis and granular proctitis, since surgical therapy is employed with benefit chiefly if not exclusively in ulcerative colitis. The role of psychosomatic factors is deposed of in four lines. The best and indeed the major portions of the work deal with the technical aspects of operative procedures, the risks, the complications, and the great importance of preoperative and postoperative management in effecting useful results. The large number of colored reproductions of microscopic sections is a notable feature of the volume which will appeal generally to surgeons but which should be in every reference library and in the hands of all working in the field of gastroenteric disease.

A Curriculum for Schools of Medical Technology. Edited by Israel Davidsohn, M.D., Professor of Pathology and Chairman of Department, Chicago Medical School; Director of Laboratories and Pathologist, Mount Sinai Hospital; Director of Research, Mount Sinai Medical Research Foundation, Chicago, and Kurt Stern, M.D., Director, Blood Center of Mount Sinai Medical Research Foundation and Hospital; Assistant Professor of Pathology, Chicago Medical School. Third Edition. Price, $3.00. Pp. 122. Registry of Medical Technologists of the American Society of Clinical Pathologists, Muncie, Ind., 1953.

This curriculum is intended for a guide in the training of medical technologists in schools approved by the American Medical Association and the American Society of Clinical Pathologists. The third edition is based on the same principles as the earlier editions but has taken into consideration the tremendous expansion of laboratory work during the ten years since the last edition.

The various chapters are devoted to the main subjects of clinical pathology: urinalysis and clinical microscopy, basal metabolism, electrocardiography, hematology, histologic technique, blood bank procedures, clinical chemistry, bacteriology, parasitology, and serology. There is a general discussion of the various methods with required reading assignments and recommended collateral reading. The book is not a laboratory manual, but valuable general information is included.

Additional chapters deal with emergency tests, laboratory records and reports, examinations, and audiovisual teaching aids. Appendices give outlines for evaluation of the student and sample records for the course. A subject index facilitates finding individual subjects.

In addition to its value as a guide to training students, it is a valuable guide to the medical technologists who wish to review the work and brush up on new techniques.

The Biochemistry of Clinical Medicine. By William Hoffman, M.D. Price, $12.00. Pp. 681. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1954.

In his preface the author includes among his aims the clear presentation of the biochemical processes of disease from the dynamic point of view and the application of this knowledge in diagnosis, prognosis and treatment. By and large these aims are pretty well fulfilled, but, as might be expected in a volume of this scope, there are a number of defects which future editions will probably correct.

The chapters on the metabolic biochemistry of proteins, carbohydrates, lipids, and electrolytes are excellent, and the correlation with clinical disturbances and their management is well developed. The discussion of diabetes, Bright's disease, cirrhosis of the liver, gout, and uremia, in all of which the author has had wide experience and special interest, is comprehensive and practical. At times the author is somewhat dogmatic, for example, on insisting on the infusion of saline or Ringer's solution as the first fluid regardless of the obvious need for blood, potassium, or other material.

As to the defects, it is curious that there is no mention of the use of corticotropin (ACTH) or cortisone in the treatment of the nephrotic syndrome; nor is there an adequate discussion of conditions associated with aminoaciduria or the recent biochemical studies on hepatic coma. The role of copper metabolism in relation to hepatolenticular degeneration is not discussed. The description of certain endocrine syndromes is quite sketchy and adds little to the value of the book. The chapter on isotopes is too brief to furnish a strong ending. Antibiotics are not mentioned in the index, and lipoproteins are not given sufficient space.

In general, however, this volume is a tribute to the author's grasp of a very wide field, and it will prove of value to all physicians interested in the integration of biochemistry and clinical medicine.

Diseases of the Skin. By George Clinton Andrews, M.D. Fourth edition. Price, $13.00. Pp. 877, with 777 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W. C. 2, 1954.

The fact that this is the fourth edition bears witness to the good qualities of this book: it is not too large, the format is attractive, the illustrations are plentiful and well reproduced, the text is to the point and clearly written, and the general introductory chapters are adequate. The discussions of therapy are reasonable and up to date and "legendary" dermatological material has been pretty well culled out.

The Microphysical World. By William Wilson, Ph.D. Price, $3.75. Pp. 216, with 14 illustrations. The Philosophical Library, Inc., 15 E. 40th St., New York 16, 1954.

This little book "is devoted to the small things in the physical world—especially to atoms, their structure and behavior," and the author tries too hard to put things in simple terms for the general reader. This he does in most interesting fashion, even though one is startled here and there by mathematical formulae obviously beyond the beginner but no doubt unavoidable. If anyone wishes to orient himself on the newer knowledge of atoms and molecules he will find this book interesting and profitable reading.

Color Atlas of Pathology: Volume 2. United States Naval Medical School of the National Naval Medical Center. Price, $20.00. Pp. 450, with 1,032 illustrations. J. B. Lippincott Company, 227-231 S. 6th St., Philadelphia 5; Aldine House, 10-13 Bedford St., London, W. C. 2; 2083 Guy St., Montreal, 1954.

A somewhat novel method of procedure is used in this book. The pages have double columns. The inner column gives a report of some specific case; in the outer are beautiful color photographs of gross specimens and microscopic sections. This is evidently the second volume of a series, and it deals with endocrinology, urology, gynecology, and dermatology. There are also brief sections in which the subject is discussed systematically.

Clinical Approach to Jaundice. By Leon Schiff, M.D. Price, $3.75. Pp. 113, with 52 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1954.

This is an excellent presentation of the jaundice problem as it confronts the physician. It presents, in a concise manner, the four methods of medical approach to jaundice: (1) history and physical examination, (2) laboratory tests, (3) roentgen examination, and (4) needle biopsy of the liver. Interpretations of the information which may be gathered by these means are offered by the author on the basis of extensive clinical experience. The limitation in diagnosis and the importance of a history and of physical examination are stressed. The text is well illustrated; the photomicrographs of specimens of liver used for biopsy are exceptionally good.

The book should be of considerable value to medical students desiring basic knowledge of a difficult clinical subject.

Symposium on Problems of Gerontology. By F. H. Bethell, M.D., and others. Price, $2.50. Pp. 141, with 27 illustrations and 21 tables. The National Vitamin Foundation, Inc., 15 E. 58th St., New York 22, 1954.

The period since the end of World War II has seen the advent and flourishing of many symposia, colloquia, and stenographic reports of work in progress. Under the auspices of various foundations a group of specialists, usually in a narrow field, are brought together to give a synoptical view of actively advancing research. While this occasionally calls to mind the breathless extravagance of the lay journals which peddle their medical miracles for the consumption of the uncritical, or the county fair side-show announcer, in general such symposia have served a very useful purpose of collecting in concentrated form a review of recent work and a preview of things to come. This present symposium falls under the latter catagory, since for the most part it points out the vast unknown terrain open for exploration in the fields of nutrition and gerontology. Both these fields have been somewhat off the beaten track and have lead many unwary clinicians into premature pronouncements and misinterpreted enthusiasm. The chief value of this particular volume, to which many distinguished contemporary American

investigators contributed, is the clear delineation of the problem and its scope and suggestions for some of the enormous amount of work which needs to be done to settle the many problems presented. It falls into the catagory of raising questions rather than presenting answers.

Transactions of the Thirteenth Conference on the Chemotherapy of Tuberculosis of the Veterans Administration, Army, and Navy, Feb. 8 to 11, 1954. Price, given on request only. Pp. 442, with 44 graphs. Veterans Administration Central Office, Washington 25, D. C., 1954.

The proceedings of the well-known cooperative study of the chemotherapy of tuberculosis of the Veterans Administration, Army, and Navy, meeting for the 13th time since December, 1946, are recorded herein and cover 60-odd papers. The volume is available in most medical libraries, but it is not purchasable in the ordinary manner.

Subjects are grouped as follows: Pulmonary tuberculosis, meningeal and miliary tuberculosis, laboratory studies, surgery in pulmonary tuberculosis, newer antituberculous drugs, extrapulmonary tuberculosis, and nontuberculous pulmonary disease. Collectively, the field of the chemotherapy of tuberculosis is extensively covered.

Of especial interest are the results of two cooperative studies, those of the Veterans Administration-Army-Navy and that of the Public Health Service, the former documented with 85 pages of tables which afford a wealth of data to the close student of the subject. In general it is found by both groups that treatment with streptomycin and *p*-aminosalicylic acid (PAS), isoniazid and streptomycin, and isoniazid and PAS are approximately equally effective. One report of treatment with all three drugs simultaneously did not demonstrate superiority.

Pyrazinamide and isoniazid therapy is reported by six groups of investigators and appears to possess considerable promise despite some threat of serious hepatic toxicity. No other new drug of promise has recently been reported. Addition of isoniazid has improved the outlook in miliary and meningeal tuberculosis. In all forms of tuberculosis prolonged drug therapy has improved the prognosis.

The volume is meaty and well edited. A detailed alphabetical subject index would add to its value. To the student of phthisiology close study of its contents is well rewarded and is well-nigh indispensable in these days of rapidly changing developments.

Acute Renal Failure. By Arthur Grollman, Ph.D., M.D. Price, $4.00. Pp. 22. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1954.

This small volume covers the subject of acute renal failure in a remarkably condensed and clear discussion. The pathology of tubular necrosis is described in terms of Oliver's recent studies. The discussions of physiological mechanisms, clinical course, and treatment follow standard current views. The prophylactic treatment in terms of maintenance of circulatory and electrolyte status is appropriately emphasized. Intermittent peritoneal dialysis is given preference among the artificial measures in substituting for renal function. The indications for the use of artificial measures are limited to the treatment for general edema from overhydration, pulmonary edema from left ventricular failure or other causes, high serum potassium concentration, and severe acidosis or intoxication by diffusible drugs. Full details are given for the procedure and the type of solution used in irrigation.

The bibliography is adequate although largely limited to the last ten years.

Fifty Years of Medicine. By Lord Horder. Price, $2.50. Pp. 70. Philosophical Library, Inc., 15 E. 40th St., New York 16, 1954.

This book is composed of three semiautobiographical lectures delivered by the grand old man of British medicine at the Royal Institute of Public Health and Hygiene in Harben, December, 1952. Either physician or layman will find the hour or so required to read this small volume an informative and inspiring experience.

As he unfolds in broad, rapid strokes the panorama of medical progress of the past fifty years, with only incidental references to his own activities, one is impressed by the immense and varied contributions made by this medical giant to both the scientific and the humanitarian developments of his profession.

Starting at the turn of the century, when the discoveries of Pasteur, Koch, von Behring, and other microbiologists had brought a new interest in exact laboratory methods to the practice of medicine, Lord Horder began his career as a young consultant who "took the

bench to the bedside." He recounts his exciting experiences in doing blood cultures and lumbar punctures in the homes of the patients. From this intense interest in the individual patient, an interest which never lagged, his activities broadened to include leadership in many phases of British medicine. Among his extraordinary list of activities he became first honorary member of the first Association of Clinical Pathologists, advisor to the Minister of Food during the war years, first president of the British Association of Physical Medicine, Chairman of the Empire Rheumatism Council and Physical Medicine, Chairman of the Nursing Reconstruction Committee of the Royal College of Nursing, Chairman of the Noise Abatement League—to name but a few positions of influence.

American physicians will be particularly interested in his thoughtful and inflexible opposition to the makeshift and, for the most part, inefficient type of socialized medicine now being practiced in England. To the young man interested in a quick look at the wide vistas opening up before him in medicine or to the older man who wishes to regain his perspective this charming little volume is highly recommended.

Films in the Cardiovascular Diseases: Survey, Analysis, and Conclusions. By David S. Ruhe, M.D.; Adolf Nichtenhauser, M.D.; Leo L. Leveridge, M. D.; Henry J. Weintraub, M.D., and Norton M. Luger, M.D. Price, $1.50 and $2.50. Pp. 128. Medical Audio-Visual Institute of the Association of American Medical Colleges, published jointly with the American Heart Association, 44 E. 23rd St., New York 10, 1953.

This booklet is in essence a catalog and presents a portion of the results of the effort of the Medical Audio-Visual Institute to discover and evaluate medical motion pictures. A review of 31 films dealing with a wide range of subjects in the cardiovascular field is presented. Subject matter includes embryology, physiology, congenital anomalies, effects of electrolytes, angiocardiography, electrocardiography, and therapy both medical and surgical. The review of each film states the intended audience and the author's name and availability of the film, and gives a summary of content and appraisal of content, presentation, and effectiveness and the reviewer's estimate of its usefulness. In addition, 113 films, not reviewed, are listed. Some of these are available, others not. A list of 35 distributors of cardiovascular films is included. Teachers and research workers in the cardiovascular field will find this catalog valuable in planning audiovisual teaching programs.

Practical Electrocardiography. By Henry J. L. Marriott, M.D. Price, $5.00. Pp. 1715 with 115 illustrations. The Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1954.

This small book on electrocardiography is an attempt to simplify the subject for the beginner. It has succeeded as well as any previous attempt, as it is difficult to get completely away from the intricacies and complexities of the subject. It is an excellent book for the beginner. The text is simple, brief, and complete. Illustrations are numerous and well situated with reference to the location of corresponding text information.

Effect of ACTH and Cortisone upon Infection and Resistance: Symposium of Section on Microbiology of New York Academy of Medicine. Edited by Gregory Shwartzman, M.D. Price, $5.50. Pp. 204, with many illustrations. Columbia University Press, 2960 Broadway, New York 27, 1953.

This is the sixth of the distinguished series of symposia on the study of infectious processes arranged by the section on microbiology of the New York Academy of Medicine. In line with present-day thinking, in which the emphasis has shifted from preoccupation with the discovery of the causative organism to intense interest in the response of the host and the factors which modify it, the papers probe deeply into the action of the adrenal cortex hormones under the stimulus of microbial invasion. It is surprising that, in a field in which previous knowledge was practically nonexistent, 31 active investigators approaching the problem from different angles should come up with findings that are in general agreement.

Extensive studies with experimental animals corroborate the clinical observation that the administration of corticotropin (ACTH), cortisone, and hydrocortisone greatly diminishes local inflammatory and general systemic manifestations of infection while at the same time allowing microbial invasion and proliferation to proceed at a greatly accelerated rate, with

consequent increased morbidity and mortality. This holds true apparently for all types of infection. For example, in the present report experiments demonstrating the phenomenon in various coccal and bacterial diseases, trypanosomiasis, syphilis, malaria, influenza, poliomyelitis, and many others are reported.

The data point to many mechanisms by which the increased susceptibility may be mediated. Abundant evidence confirms the absence of typical inflammatory response, including inhibition of phagocytosis, decrease of capillary permeability, decreased mobilization and activity of macrophages, and decrease of fibrogenesis. In animals pretreated with corticotropin or cortisone there appears to be inhibition of the formation of antibodies, but there is no general agreement that preformed antibodies are destroyed by the hormones. It would appear from the bulk of the material presented, however, that inhibition of reticuloendothelial activity is probably the most important of the mechanisms so far discovered.

The practicing physician is puzzled over the question as to whether the adrenal hormones should be used in combating the shocklike state accompanying overwhelming infections with their associated toxemia. The unequivocal answer will not be found in this volume. One would conclude that most of the contributors to this symposium would consider hormone therapy under these conditions very risky.

For thoughtful readers, practitioners, and medical investigators alike, this book will prove to be a mine of useful information, with proved fact and speculation well delineated.

Surgical Forum: Proceedings of the Forum Sessions Thirty-Ninth Clinical Congress of the American College of Surgeons, Chicago, October, 1953. Price, not given. Pp. 752, with many illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W. C. 2, 1954.

In this volume, as in previous volumes, are presented the papers read at the surgical forum of the American College of Surgeons. For the most part, they contain data gathered in the surgical laboratory, although a few are reports on clinical research.

Basic physiological problems are the subject of investigation, although some papers do deal with new surgical techniques. The volume is well organized into sections including cardiovascular, pulmonary, and gastrointestinal problems; burns and renal function; shock, nutrition, and electrolytes; wounds, infections, antibiotics, and anesthesia; plasma expanders, and cancer and steroids. Over 125 papers are included, and it seems almost impossible for a physician, be he internist or surgeon, to fail to find material of real interest in this volume.

Pregnancy Wastage. Edited by Earl T. Engle, M.D. Price, $8.50. Pp. 266, with 82 illustrations. Charles C Thomas, Publisher, 301 E. Lawrence Ave., Springfield, Ill., 1953.

The majority of the readers of the ARCHIVES will not be interested in this volume, and therefore it will not be reviewed in detail. Data on a wide variety of subjects related to "fetal wastage" are presented, with discussion of each paper by well-known investigators in the field. For those interested in the field of abortion, stillbirth, and neonatal death there will be much of value. For the investigator, clinician, or pathologist interested in fertility, abortion, prematurity, stillbirths, and Rh incompatabilities there are likewise many data, some opinion, and a good deal of interesting discussion.

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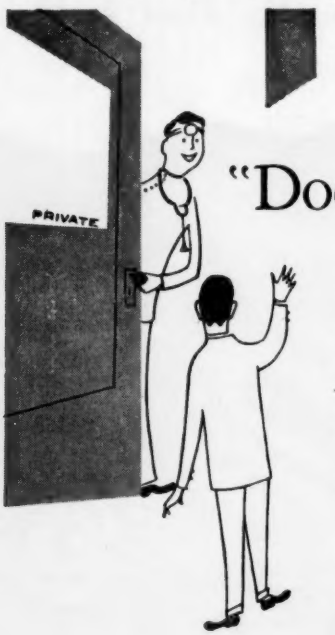

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1. Wood, H. F., and McCarty, M.: Am. J. Med. *17*:768 (Dec.) 1954. 2. Adams, F. H.: J.A.M.A. *156*:1319 (Dec. 4) 1954. 3. Stollerman, G. H., et al.: Am. J. Med. *15*:645 (Nov.) 1953. 4. McEwen, C.: M. Clin. North America (Mar.) 1955, to be published. 5. Elster, S. K.; Wood, H. F., and Seely, R. D.: Am. J. Med. *17*:826 (Dec.) 1954.

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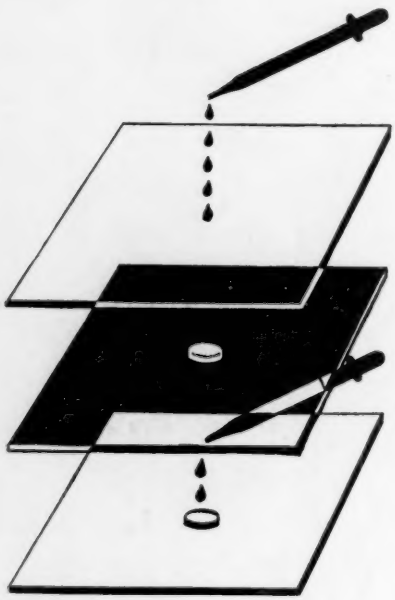

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